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A Tracer Technique to Measure Deposition of Stack Emissions

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Introduction

Tracers, materials having a readily measured distinctive property, have long been used in following wind streams in the atmosphere. Several gaseous materials have been used as tracers in meteorological studies: tritiated ethane by Tracer Laboratories; sulfur dioxide during tests on Project Prairie Grass in Nebraska; mercury vapor by Hemeon and Haines at the Industrial Hygiene Foundation; Freon at the Argonne National Laboratory.

Particulate materials have enjoyed widespread popularity as tracers; primarily fluorescent pigments such as zinc-cadmium sulfide[1] and zinc sulfide[2] dispersed as powders. With these methods, the tracer particles are caught on filters and counted microscopically under illumination with ultraviolet light. A stack gas-tracer technique employing finely divided antimony oxide powder has been reported by Haines, Cember, and Hemeon.[3] Analysis is based on neutron activation of the collected samples and measuring the induced radioactivity of the chemically separated antimony. This method provides the sensitivity attainable with radioactive tracers, yet avoids their physical and psychological hazards.

A meteorological tracer technique using the fluorescent uranine dye dispersed in a water solution by pneumatic nozzles was developed by Robinson, MacLeod, and Lapple.[4] This method avoids the tedious counting required by the fluorescent powder methods, allows the use of simple, inexpensive, and automatic sampling equipment, and provides easy dispersion, high sensitivity, and rapid analysis.

All of the tracer methods mentioned above were designed to measure atmospheric diffusion or the "dilution capacity of the atmosphere". Even the particulate tracers were purposely dispersed to have Stokes diameters of approximately five microns or less so that they would behave similarly to gas molecules. Where the downwind or ground concentration of gaseous industrial emissions is of interest, most of these techniques could be employed. Indeed, many industrial plants emit materials that satisfy the basic requirement of a tracer, i.e., that it be unique in the area to be studied and possess a distinctive property.

To apply a tracer technique to measure the deposition of particulate industrial emissions, however, requires generating a particulate tracer whose size consistently duplicates that of the particulate emission to be studied. Estimation of deposition rates from measured concentrations of gaseous tracers is beset with uncertainties, as indicated in a study reported by Duffee, Schulz, and Ungar.[5] In addition to close control of particle size, a deposition tracer method must meet the requirements that it be inexpensive, provide ease of dispersion, use nontoxic materials, and allow rapid analysis and high sensitivity. With the exception of particle-size control, the uranine dye technique reported by Robinson, MacLeod, and Lapple satisfies these requirements and was used in the development of the method described herein.

Generation and Size Control of Trace Aerosol

A liquid atomizing technique was selected to generate a tracer aerosol which has the same settling characteristics as those of the emitted material. The particle size of a tracer material dispersed as a solution can be controlled by varying one of the three parameters: orifice diameter, atomization pressure, or concentration of solution. Most generally, a pneumatic atomizing nozzle will be required to produce particles of sufficiently small size.

With a simple nozzle, pressure has little effect on the particle-size distribution of the aerosol produced as the particle size is approximately inversely proportional to the fourth power of pressure. The orifice diameter also has little effect; it controls dissemination rate primarily. The parameter having the greatest effect on the particle size of the resulting dried tracer is the

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concentration of the solution to be atomized. A droplet containing one per cent tracer at the time of atomization will dry to a particle diameter which is 22 per cent of the diameter of the original droplet; a droplet containing 10 per cent tracer will dry to a final diameter of 46 per cent of the original. If this relationship and the characteristics of the atomizing nozzle are known, it is possible to produce a tracer aerosol which will duplicate particle-size consist for almost any industrial emission.

The particle-size distribution of the tracer aerosols for this study was determined with a cascade impactor. This device classifies the aerosol using inertial forces and determines particle size as equivalent to a "Stokes" settling diameter. A complete description of the development and operating characteristics of the cascade impactor used can be found in the paper published by Mitchell and Pilcher.[8] As it is not possible to select the parameters which affect the particle-size distribution of the tracer aerosol before the solution is atomized, they are approximated and the resulting size distribution is measured. After the size distribution has been obtained, it is possible to vary one of the parameters to give the final desired size distribution.

Uranine as a Tracer

Nature and Analysis

Uranine, the disodium salt of fluorescein, is a reddish-orange powder used as a food color and as a sea marker. It is nontoxic, readily available, and comparatively inexpensive at a price of $2 per pound. It is soluble in water, forming a deep-red color at a concentration of 10 per cent, changing to yellow-green in more dilute concentrations. Solutions of uranine absorb blue light between the wavelengths of 4400–5200 A and have a brilliant yellow fluorescence between 5100 and 5900 A. This strong fluorescence permits quantitative measurement of minute quantities of uranine by simple fluorescence analysis. All uranine samples collected during this investigation were analyzed by means of a Photovolt Meter, Model 520 M.

Uranine solution concentrations of up to 0.1 microgram/milliliter yielded a linear response of the instrument. At concentrations of 10^{-8} gm/ml or less, the instrument response was nonlinear. By calibrating the meter with uranine solutions of known concentrations, it was determined that samples containing a minimum of 10^{-10} gm of uranine could be detected.

Cascade impactor slides and filter samples were placed in wide-mouth jars and washed with 20 ml of distilled water. The resulting solution was poured into a cuvette and inserted into the fluorescence meter. The background fluorescence level of the water and filters were checked during each analysis and proved to be negligible.

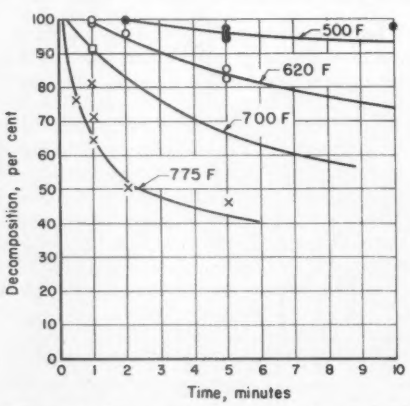

FIGURE 1. Decomposition of uranine with time at various temperatures.

Decomposition Temperature

Uranine is unstable at high temperatures. Therefore, before the method could be evaluated in field tests which could require injection of the uranine aerosol into a hot stack gas, the degradation of uranine at high temperatures was investigated. The determination of uranine decomposition with temperature was accomplished with the aid of a laboratory furnace. Ten microliter droplets containing 10 milligrams of uranine per milliliter were placed on an aluminum foil of ¾-sq-inch area and inserted into a controlled-temperature furnace for a specified length of time, between ½ and 60 minutes. After exposure was complete, the foil and sample were quenched and washed in 100 milliliters of distilled water for analysis of remaining fluorescence.

Figure 1 gives the results as a plot of decomposition of uranine with temperature for various periods of exposure.

The evaporation rates of the water surrounding the uranine particles were computed. It was assumed that during the initial heating phase the droplet acted as if it were a solid body. This allowed the application of the theory developed for the transient heating of a solid sphere. Calculations were carried out in order to determine the time for the surface to reach the boiling point of water at the stack pressure (considered approximately equal to ambient). The evaporation rate of the water was then computed.

The temperature rise of the uranine as a function of time measured on the completion of evaporation was then calculated. The calculations indicated that the time required for the evaporation of the water at high stack temperatures is negligible compared to the stack residence time. For example, the total time required to evaporate the water from the 33-micron particle is 11 milliseconds for a stack-gas temperature of 1000 F and 14 milliseconds for a stack-gas temperature of 900 F. Even at stack-gas temperatures of approximately 600 F, the evaporation time is considerably less than the stack residence time. Hence, 600 F appears to be a realistic temperature for injection of uranine into stack gases.

Decomposition of Uranine Solutions on Exposure to Atmosphere

Uranine in weak solutions is sensitive to light as well as to temperature. A common method of measuring deposition rates of particulates is by means of dust-fall jars, usually containing water or a mixture of water and an antifreeze, commonly alcohol. The use of this sampling technique with a uranine tracer would result in the formation of a very weak uranine solution in the dust-fall jar, and the solution would be exposed to sunlight. During a 2-month period, two sets of uranine solutions, together with controls, were utilized to determine the feasibility of collecting uranine tracer in wet dust-fall containers.

Three different solutions were used for this evaluation: water-uranine containing 0.1 microgram of uranine/milliliter; water-alcohol-uranine mixture; and distilled water. These solutions were set on the roof of a laboratory building and exposed for approximately one month. Identical controls were sealed and kept dark in the laboratory. Stainless steel dust-fall containers four inches in diameter and eight inches deep were used.

Figure 2 shows the results plotted as decomposition or loss of fluorescence as a function of time for each exposure sample. During the analysis, corrections were made for change in volume of solution resulting from addition by rainfall or loss by evaporation. The fluorescence of the controls did not vary during this study but 10 per cent loss of fluorescence of the exposed sample occurred in from 12 to 30 hours. A 50 per cent loss developed in from 2 to 7½ days. After 26 days, approximately 86 per cent of the fluorescence vanished. The loss in fluorescence in the dust-fall jars containing alcohol, which absorbs ultraviolet energy, was more gradual and less pronounced.

Field Tests

Emissions from 250- and 300-foot stacks at a power plant were used for this study. On the first, the flue gases passed through mechanical collectors while the other stack was equipped with both mechanical and electrostatic collectors. Tracer sampling stations were located in a sector of 135 degrees from the plant and were situ-

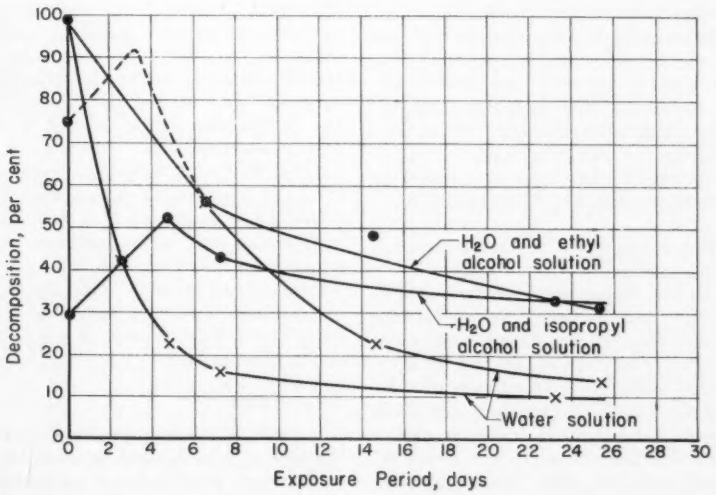

FIGURE 2. Decomposition of uranine solutions during exposure to atmosphere (solution 0.1 microgram uranine per ml).

TABLE I

Summary of Stack-emission Measurements

Fly-ash collection	Mechanical and electrostatic	Mechanical
Sampling period, min	120	120
Burning rate of coal fired, lb/hr	116,500	95,000
Stack area, sq ft	205	143
Gas flow, lb/hr	1,610,000	1,299,000
Gas velocity, ft/min	2,560	3,170
Gas flow, cfm	526,000	452,900
Gas sampled, lb	85.44	99.02
Dust sampled, lb	0.0628	0.26
Gas temperature, F	309	367
Excess air, per cent	48	44
Dust emitted, lb/hr	1,180	3,410
Dust loading, lb/1000 lb flue gas (as sampled)	0.736	2.62

ated at from ¼- to 2-mile distances. Wind records were obtained from an Aerovane located on plant grounds. Observations of plume direction were also recorded. The observed plume in the sampling area was often as much as 90 degrees away from the direction of the plume leaving the stack.

Stack Sampling[7]

Stack emissions were measured in accordance with the *ASME Power Test Code on Determining Dust Concentration in a Gas Stream, 1957*. Twenty-four points were sampled in each stack. Each point represented a 90-degree sector of six equal areas and was located on one of two traverse directions on 90-degree axes.

Stack-dust sampling apparatus, including venturi meter, pitot tube, and thermocouple, was used in establishing isokinetic sampling conditions. Dust samples were collected in Orlon filter bags and glass-wood absolute filters. Two swivel joints in the sampling probe made it possible to traverse a large-diameter stack while operating from a circular stack platform 28 inches wide.

Table I is a summary of the stack emission measurement.

Both screen and Bahco analyses were used in determination of particle size of fly ash emitted from the stack. The Microparticle Classifier determined size as a function of settling velocity. A mass-median-diameter of 14 microns was determined for the fly ash emitted from the stack containing just a mechanical collector, and a 4-micron mass-median-diameter was measured for the stack equipped with the mechanical-electrostatic collector.

Tracer Calibration

After sizes of the fly ash were determined, two pneumatic spray nozzles were used to generate the trace aerosol. One gave a mass-median particle diameter of 6 microns and the other a mass-median particle diameter of 22 microns. As uranine has a density of 1 and fly ash a density of 2.23, it was necessary to use a larger mass-median diameter for the uranine to obtain equivalent terminal settling velocities for the tracer and fly ash.

Figure 3 is a graph of the range of equivalent particle sizes obtained for both the fly ash and tracer. This plot has been adjusted for density differences but not particle shape. The tracer size distributions were measured by a cascade impactor with a cutoff size of 16 microns for the initial stage. Hence, the percentages of particle sizes for the tracer above 16 microns are extrapolated values and the apparent divergence of tracer and fly ash for the mechanical unit above 30 microns may not be real. The calibration of tracer and fly ash was designed to equate mass-median diameters, as the method yields measures of mass deposition rather than number of particles. Mass-median diameters of fly ash and tracer vary by 3 to 4 microns, a negligible difference.

Calibration of the dry tracer particle sizes was accomplished by spraying the uranine solution near the top of a closed room 35 feet high and sampling with a cascade impactor[5] near the floor. The sprayed solution filled the room and the water evaporated rapidly so that dry particles were collected at the sampling level. Amounts of uranine collected on the various stages of the calibrated cascade impactor gave the size distribution of the dry uranine. This procedure was used in both spray nozzles until the particle-size ranges desired were obtained. It was determined that a 10 per cent solution of uranine in water would be suitable for both stacks. A spray nozzle with a delivery rate of 15 gallons of solution per hour was calibrated for use on the unit with mechanical collector by providing a liquid and atomizing air pressure of 40 psig. Liquid pressure of 40 psig and atomizing air pressure of 60 psig were used for the other unit. This nozzle sprayed at a rate of 5 gallons of solution per hour.

Tracer Dispersal

Initially, uranine solution was sprayed through a hole located just above the stack inlet during dust-emission measurements. This was done to investigate the feasibility of emit-

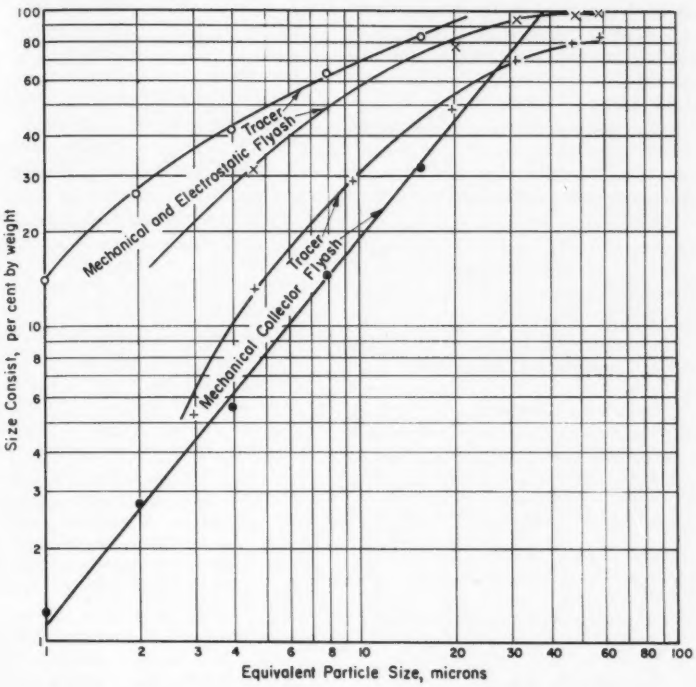

FIGURE 3. Range of equivalent particle sizes of fly-ash and tracer.

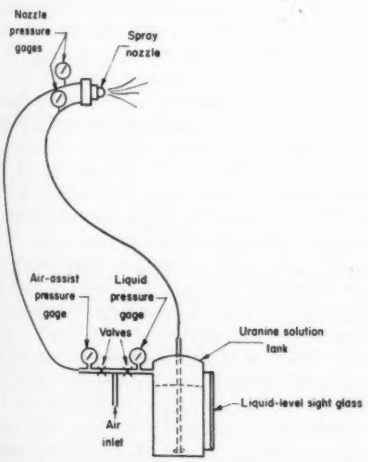

FIGURE 4. Sketch of uranine dispersion equipment.

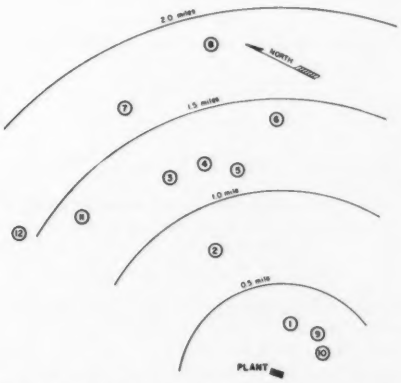

FIGURE 5. Map showing field sampling locations

ting a tracer at a low level in the stack. Dust-emission measurements showed that only about 10 per cent of the uranine reached the sampling location, halfway up the stack. This loss was due, in part, to clogging of the nozzle by fly ash. As a result, it was decided that the tracer would be emitted into the stack plume at a location about five feet above the top of the stack. Figure 4 is a sketch of the equipment used for dispersing uranine. Pulleys and ropes were used to hoist the nozzles and extension piping into place.

To accelerate the tracer tests, instead of utilizing impaction slides or dust-fall jars, samples of the emitted tracer were obtained at various locations in the sampling area by collection on 2-inch membrane filters. Air flow through the filters was controlled at ½ cfm by use of critical-flow orifices, using Gast pumps as the vacuum source. Originally, eight air-sampling stations were selected. However, because of the variability of wind direction four additional mobile sampling locations were used as required. Figure 5 shows the 12 sampling locations with distances from the plant.

Filter samples of the dispersed uranine were collected at selected sites in the sampling area for three sets of atmosphere conditions: (1) light turbulence, where there was little or no vertical oscillation of the plume, (2) moderate turbulence, where the plume neared the ground but did not touch it, and (3) heavy turbulence, where the plume actually touched the ground in the sampling area. The atmosphere dilution rate

TABLE II

Summary of Tracer Results

Sampling station	Sampling time, hr	Concentration of fly-ash, Mg/m³	Deposition rate of fly-ash, tons/(sq mile)(30 days)	Recorder wind indication		Per cent of time plume observed over stations
				Direction	Velocity	
Minimum Wind Turbulence						
Run 2: Mechanical-electrostatic collector unit, tracer emission						
1	2	2.1×10^{-4}	0.007			18
2	2	Nil	Nil			17
3	2	"	"			20
4	2	"	"			20
5	2	"	"			18
6	2	"	"			18
7	2	"	"			17
8	2	"	"			17
Average					Calm[a]	
Run 3: No tracer emission						
1	1	5.6×10^{-4}	0.020			—
2	1	1.4×10^{-3}	0.046			—
3	1	1.1×10^{-3}	0.037			—
4	1	2.3×10^{-4}	0.008			—
5	1	4.2×10^{-4}	0.014			—
6	1	3.3×10^{-4}	0.011			—
7	1	8.1×10^{-6}	0.0003			—
8	1	7×10^{-4}	0.023			—
Average			0.02		Calm	
Run 4: Mechanical collector unit, tracer emission						
1	1	2.6×10^{-3}	0.49			9
8	1	8.1×10^{-4}	0.16			5
9	1	7.4×10^{-4}	0.13			50
Average			0.26		Calm	
Run 5: Mechanical collector unit, no tracer emission						
1	1	3.5×10^{-4}	0.07			—
8	1	5.3×10^{-4}	0.10			—
Run 6: Mechanical collector unit, tracer emission						
1	1	7.1×10^{-4}	0.13			5
8	1	4.2×10^{-4}	0.08			0
10	1	8.5×10^{-4}	0.15			65
Average			0.12		Calm	

TABLE II—*Continued*

Sampling station	Sampling time, hr	Concentration of fly-ash, Mg/m³	Deposition rate of fly-ash, tons/(sq mile)(30 days)	Recorder wind indication		Per cent of time plume observed over stations
				Direction	Velocity	
Medium Wind Turbulence						
Run 7: Mechanical collector unit, tracer emission						
1	1.584	1.82×10^{-2}	3.2			2
2	1.767	1.5×10^{-2}	2.6			80
3	1.0	2.5×10^{-2}	4.4			80
5	1.735	1.5×10^{-2}	2.6			50
6	1.584	2.0×10^{-2}	3.5			50
7	1.0	1.9×10^{-2}	3.4			80
8	1.0	1.6×10^{-2}	2.7			80
Average			3.2	SSW	7	—
Maximum Wind Turbulence						
Run 8: Mechanical collector unit, tracer emission						
1	2.0	4.2×10^{-2}	7.6			5
5	2.267	7.0×10^{-2}	4.5			10
11	1.75	2.9×10^{-2}	5.2			80
12	0.5	6.7×10^{-2}	12.2			100
Average			7.4	W	5	—

[a] Calm is defined as a wind velocity of less than 3 miles per hour.

was calculated by dividing the sample weight determined by fluorescence analysis, by the weight of tracer emitted during the sample period. This dilution rate was then applied to the fly ash and the rate of fly-ash deposition or fall-out at each sampling location was calculated by applying Stokes law to the calculated concentration of fly ash. It was assumed for this investigation that the particle-size distribution sampled was the same as the distribution at the point of emission.

Table II presents a summary of tracer results. Wind direction and velocity together with observations of plume direction are shown to aid in relating fly-ash concentrations obtained and the deposition rates calculated. Background fluorescence was determined for each run and, in all cases, found to be negligible. Runs 2 and 3 show the results of minimum turbulence conditions on the emission from the mechanical- and electrostatic-equipped unit. Little deposition occurred during the two hours that tracer was emitted, as shown in Run 2. In the following hour, Run 3, an increase of dust fall occurred. This increase was attributed to the slow mixing of air in the area during weather conditions that prevailed during the run. Runs 4 and 6 were one-hour runs with tracer being emitted only from the unit with the mechanical collector during minimum turbulence. Run 5, when no tracer was emitted, indicated the presence of tracer, attributed to the "lingering" of tracer emitted during Run 4. Runs 7 and 8, also limited to the unit with the mechanical collector, show the results of medium and maximum turbulence conditions.

Conclusions

This investigation indicates that this tracer technique is a useful tool in the quantitative measurement of the particulate deposition rate of industrial emission.

The variable decompositions of uranine solutions exposed to the atmosphere in dust-fall containers prevents the use of dust-fall jars as sampling devices. Based on the laboratory study, uranine can be dispersed as a tracer into stack at stack-gas temperatures up to 600°F without decomposition. With direct stack injection, losses of tracer in the stack must be determined for each application. In using uranine it is mandatory that background fluorescence be determined in the area to be investigated. However, the technique is not limited to uranine. Any soluble material that satisfies the requirements of a tracer can be employed with this method.

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Scientific Exhibits for American Industrial Hygiene Conference

EACH YEAR THE SCIENTIFIC EXHIBITS are an important and greatly appreciated part of the American Industrial Hygiene Conference. Applications are now being accepted for space for Scientific Exhibits to be shown at the 1961 American Industrial Hygiene Conference. Exhibits will be shown from April 10 through April 13. Forms and information may be obtained from Paul M. Giever, Chairman of Scientific Exhibits Committee, School of Public Health, University of Michigan, Ann Arbor, Michigan.

The Carcinogenic Effects of Single and Repeated Doses of 3,4-Benzpyrene

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IN the last two decades there has been an increasing interest in dose-response relationships in the study of carcinogenesis. As the spectrum of known or suspected environmental carcinogens has broadened the need has become apparent for a better understanding of the effect of small, recurring, sub-acute doses active under most environmental conditions of exposure as contrasted with a single, relatively high exposure as might result from an accident. Low level, long-term exposures are encountered in the polluted atmosphere of some of our urban areas and in the working environment of industrial plants in which exposure to physical and chemical agents is controlled to an extent barely sufficient to prevent acute impairment of health.

Most of the dose-response experiments that have been reported by previous investigators have been designed to develop methodology, to study the mechanism of carcinogenesis, to determine whether a threshold or safe level exists, to determine the relation between "initiating" and "promoting" agents or to discover the nature of the changes that occur in affected cells.

Lavik and others[1] have shown that when a carcinogen is applied to the skin of mice for a time insufficient to induce tumors the subsequent resumption of the carcinogenic stimulus will quickly evoke the formation of tumors even though two or three months intervene between the two periods of treatment. Poel[2] gave three groups of mice an initial exposure of 3,4-benzpyrene by skin painting. One of the groups was given a second series of treatments immediately, but for the second group the treatment was delayed 52 weeks. The third group received only the initial treatment. The treatment was effective in producing carcinomas in both of the groups receiving the secondary treatment, but no carcinomas occurred among the third group of mice. He concluded that the process of carcinogenesis is directly dependent on the dose level and duration of exposure as well as on the potency of the carcinogen.

In a quantitative study of skin carcinogenesis in mice Cramer and Stowell[3] determined the effect of varying doses of methylcholanthrene. The dose administered by painting was varied by increasing the interval between paintings and by reducing the number of paintings. In a series in which the painting was continued until all surviving mice had developed cancer the period from the start of the treatment to the development of skin cancer was smaller for the shorter intervals between paintings. Relative to the total amount of methylcholanthrene applied the longer intervals between paintings were more effective in the production of cancers. It appears, though, that the same response may have been obtained if painting had been discontinued earlier, which would result in the administration of a smaller total dose. In another series in the experiment marginal doses were administered for limited periods using different strength solutions as well as varying intervals. The authors concluded that under their experimental conditions the carcinogenic response of the skin of mice to a small total dose of methylcholanthrene is remarkably constant, whether the carcinogen is applied continuously or discontinuously.

Saffiotti and Shubik[4] compared the effects of administering 9,10-dimethyl-1,2-benzanthracene (DMBA) by painting in a single application and in repeated doses. Mice in the first group received a single painting of one per cent DMBA while those in a second group received paintings of 0.01 per cent DMBA twice weekly. After 100 paintings the animals of the second group had received the same total amount as was given in the single painting in the first group. The second treatment was effective in producing cancers while the first was not. They suggested that small, repeated doses are more effective than large, single applications, irrespective of total quantity.

Wynder and coworkers[5] studied dosage and response by painting mice with an acetone solution of 3,4-benzpyrene varying the interval between paintings and the total period of painting. Three of the groups received the same total dose and were painted three times a week. One of the

Presented at the Twenty-first Annual Meeting of the American Industrial Hygiene Association, Rochester, New York, April 25-28, 1960.

three groups was treated continuously for six months, another was treated two weeks and then omitted two weeks while the third group was painted four weeks on and four weeks off. The percentages developing cancers during their lifetime were 45, 35 and 55 respectively, but the latent period was longer when the treatment was intermittent. For other groups that were painted once, twice or three times a week for their lifetime the cancer incidence increased and the latent period decreased with the increased frequency of painting. These authors concluded that "there appears to be a minimum and optimum rate of tumor formation that is dependent upon the total amount of benzo[a]pyrene solution applied as well as on the frequency with which the applications are made".

Dose-response studies of 3,4-benzpyrene using the subcutaneous injection technique were conducted by Bryan and Shimkin[6] and Gottschalk,[7] but only single injections were made.

The present experiment was undertaken to compare the relative carcinogenic effectiveness on mice exerted by a known carcinogen when administered in a single injection with that obtained when the same total amount of material is given by repeated injection over a long period. Apart from the purely scientifically valuable information such an experiment might yield, this study was undertaken for establishing standard values on cancerous responses to a known carcinogen demonstrated in urban air pollutants, which were to be tested in subsequent bioassays.

Materials and Procedures

The animals used in this experiment were male and female C57 Black mice from the National Institutes of Health colony. They were approximately ten weeks old when first injected and were housed in stainless steel cages. Commercial laboratory animal food and tap water were available at all times.

Benzpyrene was Eastman benzo[a]pyrene (or 3,4-benzpyrene) dissolved in Eastman trioctanoin (tricaprylin).

The benzpyrene in tricaprylin was administered by injection in the nape of the neck. When repeated doses were given, the needle was inserted as nearly as possible in the same location as previously. The concentration of benzpyrene in tricaprylin was varied so that the desired dose was contained in a volume of 0.1 milliliter. Control animals received tricaprylin only.

The experiment was in two parts, which were carried on concurrently. In the first the total amount of the carcinogen, benzpyrene, was administered in a single injection while in the second part the total amount to be given was divided into twelve equal parts given monthly. In each group there were 36 male and 36 female mice at the beginning of the experiment. The amount of benzpyrene per injection ranged from 0.008 milligram (mg) to 0.5 mg when given as a single dose and from 0.002 mg to 0.04 mg (or total doses from 0.024 mg to 0.5 mg) when administered in repeated doses. The amount of benzpyrene administered in each group of animals is shown in Table I.

The mice were observed for the presence of tumors and were killed when a firm mass approximately one centimeter in diameter was found in the region of the injection. A postmortem examination was performed on all animals except a few that were cannibalized or badly decomposed. Tissues which were grossly abnormal were examined histologically.

Results and Observations

The tumors which occurred at or near the site of injection were firm in consistency and usually well delineated from the surrounding tissue. Histological examination revealed they were sarcomas, usually of the fibrosarcoma or spindle cell type, although one squamous cell carcinoma occurred in a test animal. In only two cases more than one sarcoma was found in a single animal, but it is possible that multiple tumors may have been found more frequently if the animals with tumors had been permitted to live. In addition to the sarcomas at the site of injection a few tumors occurred at other sites in the test ani-

TABLE I

Subcutaneous Injection of Benzo[a]Pyrene in Mice in Single and Repeated Monthly Doses

Number of monthly injections	Amount of benzpyrene per mouse, milligrams	
	Per injection	Total
1	(controls)	(controls)
1	0.008	0.008
1	0.013	0.013
1	0.025	0.025
1	0.05	0.05
1	0.10	0.10
1	0.25	0.25
1	0.50	0.50
12	(controls)	(controls)
12	0.002	0.024
12	0.004	0.05
12	0.008	0.10
12	0.02	0.24
12	0.04	0.48

36 male and 36 female C-57 black mice per group.
Vehicle: 0.1 ml tricaprylin per injection.

TABLE II

Tumors in Mice Injected with Varying Amounts of Benzo[a]pyrene in Single and Repeated Monthly Doses

Total dose mg		Number of Mice with Tumors at Site of Injection and Number of Survivors after Specified Intervals								Per cent of total animals with tumors at site
		Cumulative No. of mice with tumors/No. survivors								
		3 mo.	6 mo.	9 mo.	12 mo.	15 mo.	18 mo.	21 mo.	24 mo.	
Controls	Single	0/69	0/68	0/63	0/39	0/16	0/6	0/5	0/0	0
Controls	Monthly	0/71	0/67	0/46	0/31	0/17	0/2	0/2	0/0	0
0.008	Single	0/71	0/70	0/67	0/54	0/37	0/18	0/9	0/0	0
0.013	Monthly	0/70	0/69	0/68	0/61	0/47	0/13	0/8	0/0	0
0.025	Single	0/71	1/68	6/62	6/50	7/30	7/12	7/7	7/0	10
0.024	Monthly	0/69	0/68	0/66	1/62	1/50	2/34	2/24	2/0	3
0.05	Single	0/72	3/68	5/65	7/47	7/31	7/13	7/5	7/0	10
0.05	Monthly	0/72	0/71	2/69	4/62	4/47	5/33	7/13	7/0	10
0.10	Single	0/71	17/53	17/53	17/48	17/36	17/20	17/15	17/0	24
0.10	Monthly	0/72	0/69	6/61	10/52	12/45	19/31	26/12	27/0	38
0.25	Single	0/70	12/55	18/49	20/46	20/36	20/22	20/13	20/0	28
0.24	Monthly	0/71	4/67	16/52	23/42	32/26	43/9	44/6	44/0	61
0.50	Single	0/71	14/56	18/52	19/43	19/23	19/19	19/12	19/0	26
0.48	Monthly	0/70	2/68	19/51	34/31	46/12	52/4	53/2	53/0	74

36 Male and 36 female C-57 black mice in each group.

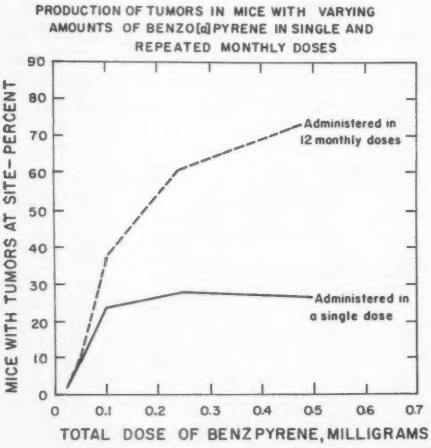

FIGURE 1. Showing total dose and percentage of mice with tumors.

mals: five lymphomas, two leukemias, one subcutaneous sarcoma near the salivary gland, one hemangiosarcoma of the liver, and one hepatocarcinoma. These are not included in the tabulated results.

A life-table analysis of the data indicates that deaths from causes other than tumors did not adversely affect the significance of the unadjusted results.

The number of mice with tumors at the site of injection and the number of survivors at the end of three-month intervals are shown in Table II. Since few deaths occurred before the appearance of the first tumor, the percentage is based on the total number of mice in each group, seventy-two, rather than the number alive at the appearance of the first tumor. No tumors occurred at the site of injection in either of the control groups or in the two groups given a single injection of 0.008 mg and 0.013 mg, respectively.

The number of tumors which occurred following a single injection increased with an increase in the amount of benzpyrene but reached a plateau with the larger doses (Figure 1). For the larger single doses, notably 0.25 mg and 0.5 mg, there was little difference in either the total number of tumors produced or the interval between the injection and the occurrence of tumors. With the smaller doses there was a difference in both the number of tumors and the time interval.

When the material was injected monthly a

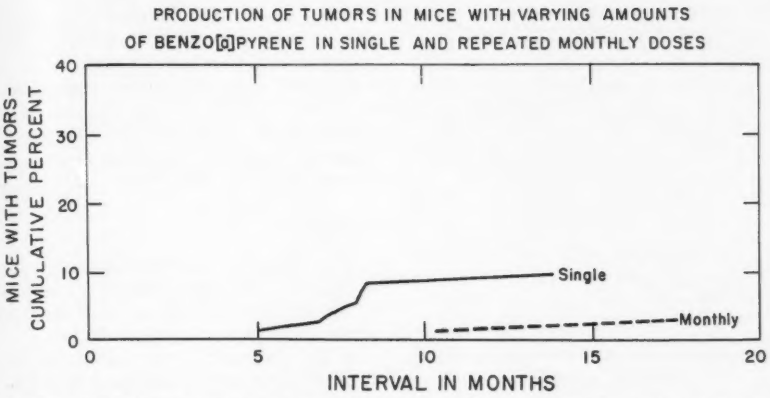

FIGURE 2. Showing percentage of mice with tumors and the interval between first injection and death. Total dose was 0.025 milligram.

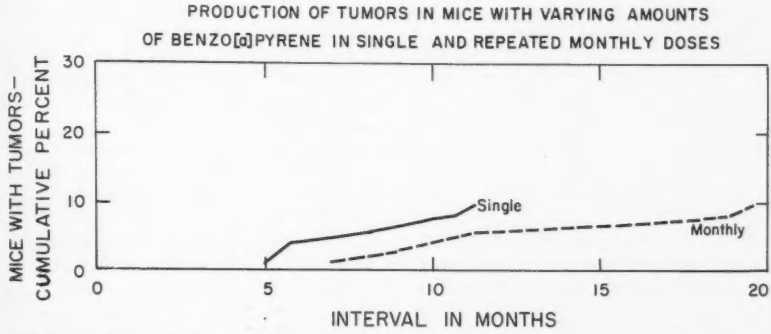

FIGURE 3. Showing percentage of animals with tumors and the interval between first injection and death. Total dose was 0.05 milligram.

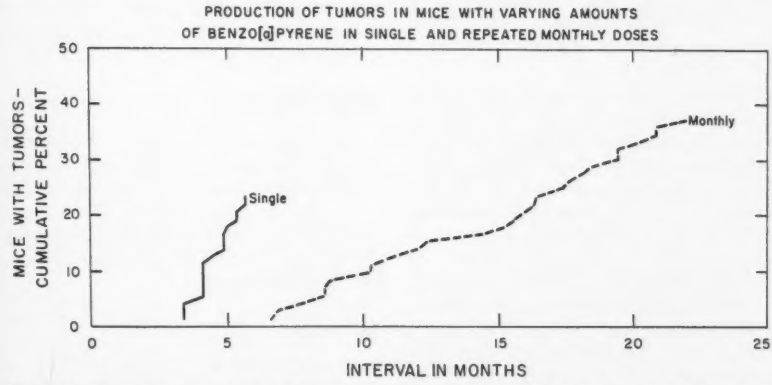

FIGURE 4. Showing percentage of mice with tumors and the interval between first injection and death. Total dose was 0.10 milligram.

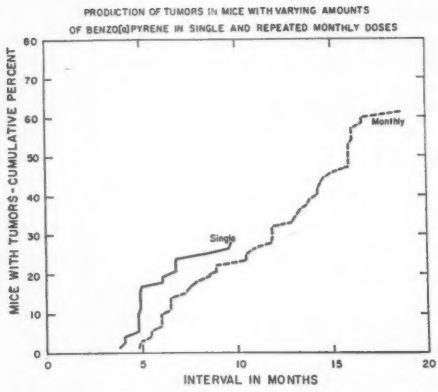
PRODUCTION OF TUMORS IN MICE WITH VARYING AMOUNTS
OF BENZO[a]PYRENE IN SINGLE AND REPEATED MONTHLY DOSES
MICE WITH TUMORS - CUMULATIVE PERCENT
INTERVAL IN MONTHS
Monthly
Single

FIGURE 5. Showing percentage of mice with tumors and the interval between first injection and death. Total dose was 0.25 milligram.

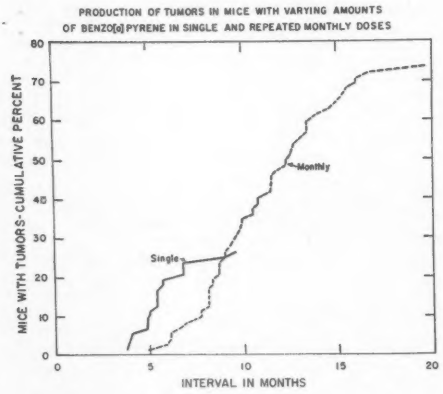
PRODUCTION OF TUMORS IN MICE WITH VARYING AMOUNTS
OF BENZO[a] PYRENE IN SINGLE AND REPEATED MONTHLY DOSES
MICE WITH TUMORS - CUMULATIVE PERCENT
INTERVAL IN MONTHS
Monthly
Single

FIGURE 6. Showing percentage of mice with tumors and the interval between first injection and death. Total dose was 0.50 milligram.

plateau did not occur in the range of doses used in this experiment. Except at very low levels, a given amount of benzpyrene produced more tumors when administered in twelve monthly doses than when given in a single injection. The difference between the carcinogenic effect produced by a single injection and that produced by multiple doses increased as the total amount of benzyprene was increased (Figures 2, 3, 4, 5 and 6). For 0.5 milligram the effect was almost threefold. Although a larger number of tumors were produced with multiple doses, there was a considerable time lag in the appearance of the tumors when the total dose was given in small increments.

From these observations it appears that the effects produced by the administration of a carcinogen are dependent not only on the amount administered but whether it is given in a single dose or in multiple doses. The carcinogenic response under these conditions, therefore, is not constant but increases when the total dose is administered in increments.

Summary

The aromatic hydrocarbon carcinogen 3,4-benzpyrene was administered to mice by subcutaneous injection in a graduated series of single and repeated monthly doses.

1. For the smaller single doses the number of tumors increased and the latent period decreased with increased amounts of benzpyrene. As the single dose was increased further a plateau was reached and an increase in the amount of benzpyrene did not increase the carcinogenic effect.

2. For repeated monthly doses a similar plateau was not observed in the dose range used in this experiment.

3. Except for the very small doses, a given total amount of benzpyrene produced a larger percentage of tumors when it was injected in twelve monthly doses than when it was administered in a single injection. For 0.5 milligram, the largest dose used, the effect was almost threefold.

4. For the repeated injections there was a longer time interval from the first injection to the first appearance of tumors than for the single injections.

5. At least for mice, a given amount of carcinogen is more effective when it is administered in small increments over a long period of time than when it is administered in a single dose. Conversely, it appears that a low-level, recurring exposure to a carcinogen is more hazardous than a single exposure to the same total amount.

Acknowledgments

The technical assistance of Mr. William Lee, Mr. Herbert Auerbach and Mrs. Ruby Walker is greatly appreciated. The histology sections were prepared by Miss Johanna Zuefle assisted by Mrs. Norma Riddick, for which we are grateful. We are indebted to Mr. Nathan Mantel for the life-table analysis.

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NEW DIVISION OF OCCUPATIONAL HEALTH

THE U. S. PUBLIC HEALTH SERVICE has reorganized its occupational health activities with the establishment of a new Division of Occupational Health effective September 1, 1960. The organization of the new Division provides for the immediate growth and more effective functioning of research, assistance, and training operations in occupational health. Dr. Harold J. Magnuson continues to guide these operations as Chief of the Division.

One of the three Branches, the States Service Branch with Henry N. Doyle as Chief will also work out of Washington headquarters. The other two Branches will function in the Occupational Health Research and Training Facility (formerly named the Occupational Health Field Headquarters) in Cincinnati, Ohio. Dr. Douglas H. K. Lee is Chief, and Dr. Lewis J. Cralley is Assistant Chief of both the Facility and the Research and Technical Services Branch. A Chief of the Training Branch is yet to be appointed.

The Facility at 1014 Broadway, Cincinnati, Ohio is being expanded to occupy most of the present building, equipment will be modernized, and additional personnel are being placed on the staff. A more forceful and expanded program is in immediate prospect to better meet the varied responsibilities of the Public Health Service in all phases of occupational health.

Determination of Manganese in Air and Biological Material

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THE increased use of organometallic compounds by the chemical industry has aroused interest in the toxicology and the industrial hygiene problems associated with their manufacture and use. One of these compounds, cyclopentadienyl-manganese-tricarbonyl has found use as an additive to motor fuels.[1] Consequently, there is considerable interest in the human absorption of the material and in methods for the determination in the air of the compound itself as well as the products of its decomposition.

Analytical efforts were directed initially toward the determination of manganese in the urine and blood of persons employed in the manufacture of cyclopentadienyl-manganese-tricarbonyl. Because of the requirement for specificity and because of past experience with the spectrochemical determination of manganese, attention was immediately given to the spectrochemical approach.[2] It soon became apparent, however, that the sensitivity of this method was inadequate and that modifications had to be introduced so that the trace concentrations normally present in the blood and urine could be determined with precision.

Dean and Cain have shown that it was possible to separate manganese, copper, and nickel from extraneous ions in solution by extracting with chloroform the complexes formed by these metals with sodium diethyldithiocarbamate.[3] With this step manganese could be extracted free from most ions and concentrated into a volume of 1 ml of solution. This extraction is the basis of the present spectrochemical method which is capable of detecting 0.05 μg of manganese per milliliter of solution. This sensitivity of detection was far superior to that possible with the periodate method (permanganate).[4] Other colorimetric methods based on reacting manganese with leucomalachite green,[5] ethanolamine,[6] or formaldoxime,[7] as well as a polarographic method[8] were investigated and discarded temporarily as lacking proper specificity or sensitivity or as being inconvenient and time consuming.

Presented at the Twenty-first Annual Meeting of the American Industrial Hygiene Association, April 25-28, 1960, in Rochester, New York.

Special Reagents

Ammonium Citrate Solution: Dissolve 400 gm of citric acid in 500 ml of distilled water. Neutralize with concentrated ammonium hydroxide to pH 8.3, using phenol red as indicator, and dilute to one liter with distilled water. Treat the solution first with dithizone in chloroform, and finally with 20 ml of 10 per cent aqueous sodium diethyldithiocarbamate, followed by repeated additions of 10-ml portions of chloroform to remove all metals, particularly manganese. The chloroform is discarded.

Acetate Buffer Solution: Dissolve 247 gm of ammonium acetate ($NH_4C_2H_3O_2$), 109 gm of sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$) and 6 gm of glacial acetic acid in distilled water. Dilute with distilled water to a volume of one liter. The pH of this solution will be approximately 6.75. For removal of trace metals, treat the solution exactly as was described above for the ammonium citrate solution.

Sodium Diethyldithiocarbamate Solution (Aqueous): Dissolve 66 gm of reagent grade sodium diethyldithiocarbamate, $(C_2H_5)_2NCSSNa \cdot 3H_2O$, in triple-distilled water. Filter through a fluted filter paper to remove insoluble matter and finally dilute to one liter. Store the solution in a polyethylene container.

Triple-Distilled Water: Double-distilled water is redistilled from a glass still.

Ammonium Hydroxide (metal-free): Bubble cylinder ammonia into ice-cooled triple-distilled water. Set the specific gravity at 0.90 by dilution with triple-distilled water.

Chloroform: Redistill from glass, then add one per cent of absolute alcohol.

Standard Manganese Solution: Dissolve one gm of manganese metal (carbon-free powder) in redistilled nitric acid and dilute to 100 ml with triple-distilled water (1 ml = 10 mg Mn per ml). This stock solution may be further diluted for use in preparing the standards used to develop the working graphs.

Buffer and Internal Standard Solution: Add 7.5 mg bismuth as the nitrate and 10 ml of redistilled nitric acid to 50 ml of synthetic urine salt solution.[9] Add triple-distilled water to dilute to 100 milliliters. The synthetic urine salt solution is previously treated with one gm of sodium diethyldithiocarbamate for every 500 ml of solution, then with 10-ml additions of chloroform to remove all of the metals. The chloroform is discarded. Solutions of sodium chloride, potassium chloride or lithium chloride, one to two per cent in strength can also be used as spectrographic salt buffers.

Cupferron Solution (Aqueous): Dissolve cupferron in triple-distilled water to make a six per cent solution (6 gm/100 ml). Store in a refrigerator.

Collection of Manganese from the Air

Manganese, as inorganic matter, may be removed from the air by the conventional methods using impingement, electrostatic precipitation or filtration through suitable fibrous media (Whatman #40 paper or Millipore filters). Vapors of organic manganese compounds may be absorbed in suitable organic solvents, preferably hydrocarbons, such as hexane or iso-octane.

Preparation of Samples

Air Samples: Dry ashing or wet digestion techniques are satisfactory for the preparation of material collected as described above. If liquids are used, they are evaporated to dryness and the residues are then ashed. The material may also be digested with a mixture of sulfuric and nitric acids in order to destroy organic matter. In the case of organometallic vapors which have been trapped in a hydrocarbon, treat the hydrocarbon absorbent with bromine until the bromine color remains for at least two minutes. Evaporate to dryness, add two or three drops of nitric acid and again evaporate to dryness. Proceed with the extraction of manganese as in the case of urine. If large amounts of iron are present, treat with cupferron and remove the iron as described in step 2 below.

Biological Samples: Place 100 ml or less of urine or 10 to 20 gm of blood or tissue into a 150-ml silica dish. In the case of urine add 10 to 20 ml of nitric acid and evaporate to dryness. Blood and tissue samples are merely dried in the dish. Ash the dried material in a muffle furnace maintained at 500°C. Ashing should be completed in one to two hours. If the ash is not clean, treat it with a few milliliters of nitric acid, evaporate to dryness and replace in the furnace. Repeat the acid and ashing treatments until a clean ash is obtained. Dissolve the ash in 2 to 3 ml of triple-distilled nitric acid and about 20 to 30 ml of triple-distilled water. Transfer the solution to a 50- or 100-ml graduated cylinder and make up to the mark with triple distilled water.

Extraction and Estimation of Manganese

1. Depending on the amount of manganese expected (not exceeding 60 μg) transfer all or a suitable aliquot of the sample into a 250-ml pyrex glass Griffin beaker and evaporate to dryness on the hot plate.

2. In the case of samples other than urine, when appreciable quantities of iron are present (in blood, liver, etc.), add 4 ml of concentrated hydrochloric acid and 10 ml of triple-distilled water. Heat to effect solution. Transfer to a 150-ml, Squibb-type separatory funnel and dilute to 40 ml with triple-distilled water. Add 5 ml of 6 per cent aqueous cupferron, shake vigorously for 30 seconds and then add 10 ml of chloroform. Shake, allow the layers to separate, and discard the chloroform layer. Shake repeatedly with 10-ml portions of chloroform until the chloroform is colorless. Repeat the treatment with cupferron until the chloroform extract shows no color. (Usually two treatments with cupferron are sufficient but additional treatments may be required when large samples are used, or when large quantities of iron are present). Withdraw the aqueous layer to a 250-ml beaker and evaporate to dryness. Step 2 is omitted in the case of samples of urine, the ashed samples being merely dissolved as in step 3.

3. Cool, then add 100 ml of triple-distilled water, 4 drops of 0.1 per cent aqueous chlorphenol red, 20 ml of ammonium citrate solution, 10 ml of acetate buffer solution and ammonium hydroxide drop by drop until pH 6.5 is reached (cherry red). The pH can be checked with "Hydrion" paper.

4. Transfer the solution to a clean 500-ml, Squibb-type separatory funnel and dilute with triple-distilled water to a volume of approximately 400 milliliters.

5. Add 20 ml of 10 per cent aqueous sodium diethyldithiocarbamate solution. Mix and shake vigorously. Add 10 ml of chloroform and shake for two minutes. Allow the layers to separate.

6. Transfer the chloroform layer to a clean 150-ml separatory funnel and repeat step 5 as long as color persists, combining all of the chloroform extracts in the 150-ml separatory funnel.

7. Wash the chloroform extracts in the 150-ml separatory funnel with 50 ml of triple-distilled

water. Transfer the chloroform layer to a cone-shaped centrifuge tube of 50-ml capacity and evaporate to dryness by immersion in a heated bath of glycerine.

8. Add six drops of concentrated nitric acid, and one ml of the salt-buffer internal standard solution to the tube. Evaporate to one ml, cool, and reset the volume at one milliliter.

9. Using ¼-inch National Carbon type L-4018 preformed cup electrodes, add 0.2 ml of the solution obtained in step 8 to each of two electrodes and dry the electrodes in an oven at 100°C. These electrodes form the positive electrodes of the 110 Volt DC arc, the counter electrode in each case being a National Carbon type L-4054 preformed rod with center post. Eastman #33 plates are used to record the spectra in duplicate. An exposure time of two minutes is employed. The plates are developed in D-19 developer. After fixing and drying, the spectra are photometered in order to obtain the ratio of the intensity of the manganese line at 2801Å to that of the bismuth line at 2898Å. This ratio is then related to micrograms of manganese by reading the value from a graph prepared by adding known amounts of manganese to a series of centrifuge tubes, evaporating to dryness, adding one ml of the buffer salt solution and photographing the spectra as described above.

Discussion

Samples may be dry-ashed as described above or they may be wet-ashed using sulfuric-nitric-perchloric acid mixtures. If the wet ashing procedure is used, heat the samples until the sulfuric acid is removed and then proceed with the analysis in the manner described above. Tables I and II illustrate the recoveries of trace quantities of manganese added to samples of urine and blood. All samples in these series were prepared by the wet digestion ashing procedure.

The method as described is specific for manganese, except for a possible interference by zinc, which yields a fairly sensitive spectral line only 0.04 Angstrom units from the manganese line. However, the amounts of zinc normally encountered in biological material do not appear to produce the spectral line of zinc. Even 100-μg quantities of zinc added to that normally present in 100 ml of urine or 10 gm of blood caused no interference.

Other metals which form complexes with sodium diethyldithiocarbamate, such as nickel, lead, bismuth, cadmium, stannous tin, copper and small amounts of ferric ions are also extracted by chloroform, but none of them interferes. Of these metals, nickel is extracted quantitatively at pH 6.5 and can be determined spectrographically simultaneously with manganese. Large quantities of iron interfere, not because of masking the spectral line of manganese, but because of the foreign ion effect on the intensities of the spectral lines of manganese and bismuth. Therefore, iron must be removed.

The buffer salt used in this work has been designed to simulate the ash of human urine and is added to all samples in an amount equivalent to 10 ml of urine. Other buffer salts such as sodium chloride, or potassium chloride may also be used, provided the concentration of bismuth is mantained at 75 μg per milliliter of buffer solution and the salt concentration is one to two per cent in strength.

In this work, as in other spectrographic work done in this laboratory, spectra are photographed through a 5-step sector. This allows for greater flexibility in photometering the spectra lines. When the section of the line in the last exposure step (step 5) is measured along with the standard line in the second step, the range of concentration which can be determined spectrographically is extended to 60 μg per milliliter of solution. Thus, with a series of calibration graphs, a single spectrum can be used to cover the range from 0.05 to 60 μg of manganese per milliliter of solution. The section of the manganese line at 2801Å in step 1 is used for the range of 0.05 to 1.5 μg of manganese per milliliter of solution, and that in step 3 for concentrations from 0.1 to 20 μg per milliliter. The section of the line in step 5 may be used for the range of 8

TABLE I

Recoveries of Manganese Added to 100-Milliter Portions of Urine

Manganese Added micrograms	Manganese Found micrograms	Average
0	0.16, 0.14, 0.25	0.18
1	1.3, 0.7, 1.5	1.2
5	5.6, 4.8, 5.2	5.2
10	12.0, 10.5, 9.2	10.5

TABLE II

Recoveries of Manganese Added to 10-Gram Portions of Blood

Manganese Added micrograms	Manganese Found micrograms	Average
0	0.27, 0.34, 0.46	0.36
1	1.4, 1.2, 1.4	1.3
5	5.8, 5.3, 5.6	5.6
10	12.0, 11.0, 8.0	10.3

to 60 μg of manganese per milliliter of solution, although greater accuracy is obtained if the analysis is repeated after the sample is diluted with a sufficient amount of buffer solution so that the section of the manganese line either in step 1 or step 3 can be used (Figure 1).

In interpreting results, it is important to know the normal levels against which the analytical findings must be compared. The expected normal concentrations of manganese in a variety of materials encountered in the practice of industrial hygiene are presented in Table III. The mean concentration for a group of subjects was found to be 2.75 μg per liter of urine and 4.58 μg per 100 gm of blood. The average concentration of manganese in city air is 0.2 μg per cubic meter, but it may range from 0.05 to as high as 5 μg per cubic meter, depending on the site and the nature of the industries near the site. The urine of persons exposed to manganese may show concentrations of several hundred micrograms per liter of urine (Table IV). The fluctuations in the subjects are illustrated by the data in Table V.

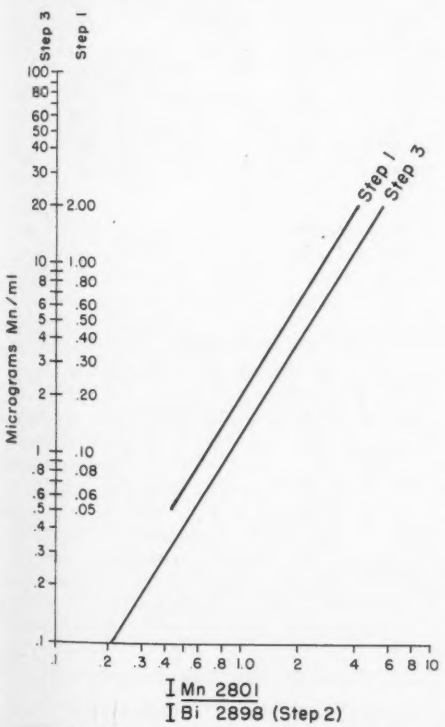

FIGURE 1. Working graphs for the spectrochemical determination of manganese.

TABLE III

Normal Levels of Concentration of Manganese in Various Materials

Material	Number of subjects	Micrograms, Mn		
		Mean	Std. Dev.	Range
Urine.....	21	2.75/liter	2.23	1.0–7.8/liter
Blood.....	12	4.58/100 gm	1.55	2.2–7.9/100 gm
Air.....	958	0.23/m³	0.43	0.01–5.70/m³

TABLE IV

Examples of the Levels of Concentration of Manganese in the Urine from Individuals Exposed to Manganese Compounds

Subject	Micrograms Mn per liter
J. C.	36.2
M. H.	78.0
A. B.	23.8
D. B.	143.0
J. G.	49.0
L. D.	64.4
J. R.	680.0

TABLE V

Fluctuations in the Concentration of Manganese in the Urine, Feces and Food

Subject	Urine			Feces			Food		
	Micrograms/liter			Milligrams/24 hours					
	No.	Mean	Std. Dev.	No.	Mean	Std. Dev.	No.	Mean	Std. Dev.
S. B.	30	4.83	2.00	28	8.59	4.97	31	6.10	2.79
F. C.	172	3.40	2.81	171	3.82	2.10	172	3.99	2.18
M. B.	21	3.23	2.45	13	2.54	3.15	21	4.43	4.82
J. S.	221	3.72	2.12	161	6.26	4.88	221	4.58	3.87

Contamination is an important consideration in applying this or any other sensitive method of analysis. Reagents must therefore be purified to remove extraneous manganese. The water and the acids used, particularly nitric acid, must be redistilled, preferably from glass, and all salt reagents must be purified by the treatments described above. Glassware and other facilities must also be scrupulously clean, if reliable results are to be obtained.

The permanganate method may be used satisfactorily for analyzing dusts collected from the industrial environment, provided the sample is of such size as to allow for the presence of at least one μg of manganese. The spectrochemical

method detects as little as 0.05 μg of manganese and therefore permits the use of smaller samples, principally of such biological material as urine, blood and tissues.

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AIHA 1961–1962 Annual Meetings

PLANS FOR THE TWENTY-SECOND ANNUAL MEETING OF AIHA are shaping up rapidly and excitingly. This meeting, to be held April 9 through 13, 1961 at the Sheraton-Cadillac Hotel, Detroit, Michigan, will initiate a number of new ideas and projects to stimulate and inspire you. There will be more general sessions and a medical session has been planned for the physician members.

There are still a few spots available on the program so all of you who have papers to offer should write at once to the Program Chairman or the appropriate Session Arranger. No need to look up the names and addresses; here they are (with corrections of several inaccuracies previously published):

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Owens-Illinois Glass Company
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Liberty Mutual Ins. Co.
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Engineering
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Los Alamos Scientific Laboratory
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Los Alamos, New Mexico

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Los Alamos, New Mexico

Radiation
Frank L. Paschal, Jr.
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Ft. Worth, Texas

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50 W. 50th Street
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The Twenty-third Annual Meeting will be held May 7 through 11, 1962, at the Lord Baltimore Hotel, Baltimore, Maryland. Plan now to be with us in 1961, and start thinking and planning for 1962.

Calibration and Evaluation of Gas Detecting Tubes

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Introduction

DEVICES for the rapid determination of atmospheric concentrations of toxic gases and vapors have long been tools of the industrial hygienist. Accuracy, reliability, portability, and ease of operation are among the desired characteristics for these instruments. Direct chemical reaction devices which use impregnated granules or gels are potentially capable of attaining these characteristics. From 1935 to the early 1950's relatively few of these devices were described in the literature. Some of these were for H_2S,[1] organic halides,[2] CO,[3] aromatic hydrocarbons[4] and SO_2.[5] Early in the last decade, Kitagawa, of the Yokohama National University, began to publish results of his experiments in devising colorimetric tubes for gas analysis. These were originally designed to detect impurities in process gas streams.[6, 7] Later, manufacturers in the United States, Germany, and Sweden began to produce a large variety of detector tubes. At this time (July 1960) there are at least seven companies producing or marketing tubes in this country.[8]

The Occupational Health Program of the United States Public Health Service was requested to evaluate the accuracy and reliability of certain of the available detector tubes. This report is based on a portion of that work, and thus is not to be taken as a comprehensive evaluation of all detector tubes on the market. Not all manufacturers are represented in this report, nor are all the tubes manufactured by any one company necessarily included. Inclusion of a given device should not be construed as an endorsement of that device by the Public Health Service. Conversely, omission of a particular manufacturer or tube does not imply disapproval. In some cases, a given tube was of no interest to this study; in others, a company may have marketed a tube after completion of our tests for the gas which it was designed to detect.

Based on work supported by the Bureau of Ships, Department of the Navy under Contract No. S 23(649B) Ser. 649B-333 dated 25 Feb. 1959.

Development of new or improved tubes, or changes in tube characteristics and calibrations may outdate rapidly a comprehensive report. To illustrate, in April 1959, the Committee on Air Sampling Instruments of the American Conference of Governmental Industrial Hygienists reported an evaluation of one manufacturer's product, "... based almost entirely upon the experience of various industrial hygiene groups in calibrating and using this equipment."[9] Of the eight types of tubes reported upon none was listed as performing according to the manufacturer's claims. Subsequently, the president of the corporation wrote the following to the Conference. "... most of the criticisms in this report refer to detector tubes ... not sold for many years. ... we are continuously developing the detector tubes. ..."[10]

In this report we shall show the characteristics of some of the tubes in the lots tested. This report will also deal with the techniques of calibration and evaluation, establishment of gas concentrations, reading of the tubes, estimation of atmospheric concentration, and the subsequent evaluation of the environment by an interpretation of the tube data. The present report will deal with certain gases only. Tubes for other gases and solvents will be discussed in a subsequent report.

Types and Description of Tubes

Some of the direct-reading colorimetric gas detecting devices available in this country are in kit form. A single aspirator is used for all tubes regardless of the contaminant. Other companies supply individual tubes and pumps for each specific gas or vapor. The type of pump and flow regulator varies with each manufacturer. Tube diameter, length and packing also differ from company to company as well as among the products of any one manufacturer.

Detector A is sold in kit form. The pump used is a bellows device with a nominal capacity of 100 milliliters. The rate of flow through the sam-

pling tube is a function primarily of the packing of the tube in that the only resistance to the flow is offered by the tube itself. Detector tubes have an outside diameter of approximately 7.5 mm and a length of approximately 75 mm. In most cases the calibration is marked directly on the tube. An instruction booklet is packed with each box of tubes.

Detector B is sold in kit form. The pump comprises a cylinder of 100 ml nominal capacity to which has been fitted a hand-honed piston. A critical flow orifice is placed between the sampling tube and the pump to provide constant flow for the greater part of the sampling period. The tubes have an approximate diameter of 4 mm and an approximate length of 85 mm. A calibration chart is supplied with each box of tubes. Where the length of a stain is used for estimating concentrations, the calibration is based on the ratio of the stained length to the total length of gel in the packing.

Device C is also in kit form. Two models are available, with or without a furnace which is used for the combustion of halogenated hydrocarbons to the halide. The pump used is a squeeze-bulb of 50 ml nominal capacity with a check valve. Flow rate through the sampling tube is a function of the tube packing. Tube diameter is approximately 5 mm; tube length, approximately 45 mm. This device is unique in that the manufacturers' calibrations are semiquantitative. The tubes indicate concentrations at the threshold limit value (TLV)[11], approximately one-half the TLV or less, and approximately twice the TLV, or greater.

Manufacturer D supplies a complete unit for each gas or vapor. In general, the pumps are squeeze bulb devices usually of 50 ml nominal capacity. Flow rate is controlled by an adjustable needle valve. The tube diameters and lengths vary between tube types.

Manufacturer E supplies only a carbon monoxide detecting tube, developed by the National Bureau of Standards.[8] A squeeze bulb pump with an adjustable needle valve is used for inducing air flow.

Type F is another carbon monoxide detector. It differs from that used in the NBS type CO tubes in that the estimate of concentration is made by measuring the length of the developed stain rather than by a color change. The pump used with this tube is a spring loaded diaphragm type of 16 ml nominal capacity.

Device G is a single tube used for the determination of tri- and perchloroethylene, and gasoline vapors. The pump used is a squeeze bulb of approximately 50 ml capacity. No flow regulating device is used.

Theory of Tube Calibration

To fully characterize indicator tubes and to predict their capabilities and limitations, certain quantitative relationships are required. The important primary variables are test gas concentration, C, and stain length, L. For increasing the tube sensitivity, the volume of the air sampled, V, is an important secondary variable. Errors caused by random variations of tube cross sectional area, A, by change in flow rate, and by temperature must be considered as well. If effects at the glass walls are assumed to be negligible the variables V and A can be combined into a single generalized variable V/A. Further, flow rate may be generalized as a linear velocity, V/At, where t is the time during which the sample is drawn.

Two types of responses are possible with detector tubes, changes in color or color intensity, or the development of a stain whose length is proportional to concentration. The reactions may be categorized into the following:

1. Incomplete sorption of the test gas.
2. Complete sorption; stoichiometric relation between quantity of sorbed gas and volume of discolored indicator which is independent of gas concentration.
3. Complete sorption; stoichiometric relation between gas and indicator which varies with gas concentration.
4. Complete sorption; non-stoichiometric relation between gas and indicator.

A theoretical analysis of each of these four limiting cases will be made. In practice tube reactions may be intermediate cases. Prediction of the response of a detector tube with varying flow rate, concentration, or sample volume may be made if one of the equations listed can be fitted to the experimental data.

Case 1: In the color tint change type of detector, the test gas is only slightly sorbed by the indicator gel. The concentration of gas in the exit stream is almost the same as in the entering mixture and a uniform color is developed. The relation may be expressed by:

$$\text{Color Intensity} = kCV$$

where k is a kinetic rate constant, C is the gas concentration, and V is the volume sampled. For a fixed flow rate V represents the time of contact of gas with indicator. Factor k is affected by temperature. It is affected by flow rate if diffusion through a gas film is a limitation of the reaction rate. If temperature, flow rate and sample volume are held constant, a scale of tints related to concentrations may be constructed. Appreciable corrections may be required if there

are deviations from the calibrating temperature and flow rate.

Case 2: This is the simplest relation among the length-of-stain detectors and the basis of the customary predictions of performance. For the assumptions made for case 2 we can equate the volume of discolored gel as proportional to the amount of test gas in the sample:

$$AL = kCV \tag{1}$$

or

$$L = kC\ V/A \tag{2}$$

Here, factor k represents the stoichiometric relationship. Temperature and flow rate would have only an indirect effect according to the change, if any, produced in the stoichiometry. A log-log calibration plot of L versus C should thus have a slope of unity.

Figure 1 contains such a plot for three nitrogen dioxide tubes of different manufacture. The curve labeled K and H refers to the data presented by Kinosian and Hubbard.[12] The slopes of these plots vary from 1.4 to 4.9. This deviation from a slope of unity is typical rather than exceptional for tubes now available, thus the assumptions for case 2 do not hold generally.

In Case 3 where complete sorption occurs and the stoichiometry is assumed to vary with the gas concentration according to the laws of equilibrium gas adsorption, we assume that a relatively sharp stain front advances through the tube. It is further assumed that the major portion of the gas is removed right at the stain front itself and that all the discolored gel upstream from the front contains test gas at the saturation concentration. Here, Freundlich's isotherm equation applies

$$x = k'\ C^n$$

where x is the mass of gas sorbed per unit mass of gel, and k' and n are empirical constants for a given gas and a given gel. A material balance equates the amount sorbed on the gel to the amount removed from the air stream:

$$k'\ C^n\ AL = k''\ VC$$

or

$$L = \frac{k''}{k'}\ C^{(1-n)}\ \frac{V}{A} = kC^{(1-n)}\ \frac{V}{A} \tag{3}$$

The value of the exponent $(1 - n)$ may be determined as the reciprocal of the slope in a plot such as Figure 1. It is evident that for a tube in which the stain length varies with flow rate, the assumptions made for Case 3 do not hold. It should be noted too that the relations for the gases shown in this figure may be more complex, as will be discussed under Case 4.

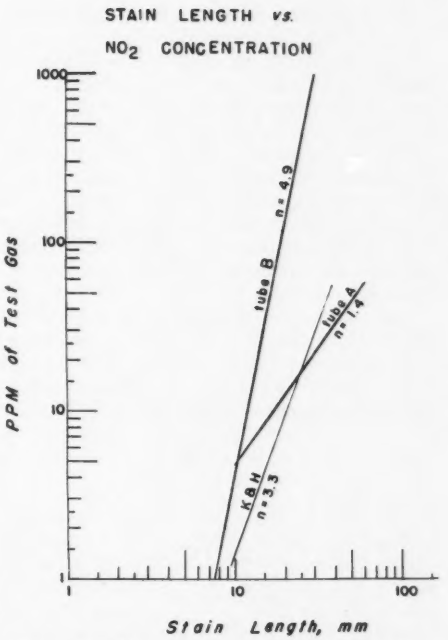

FIGURE 1. Stain length vs. NO_2 concentration.

It can be seen that if $n = 0$, no sorption occurs and equation (3) reduces to equation (2). On the other hand if good sorption occurs, and n approaches one, stain length would vary with the sample volume, but not with the concentration. This means that a fixed depth of sorbent would completely remove the test gas from a fixed volume of air regardless of the gas concentration. (The amount of gas which can be sorbed increases exactly in proportion to the concentration.) Such a gel, obviously, would be poorly suited for use in indicator tubes.

Case 4: From some experimental data it would appear that for practical length-of-stain detectors, the test gas is completely sorbed, but discolors a non-stoichiometric quantity of indicator. These are the conditions for Case 4. Here we assume that most of the gas is sorbed on the gel, but that equilibrium saturation is never attained because of the relatively small sample volume and the relatively high flow rate. Thus the concentration of sorbed gas in the stained gel diminishes from the inlet end to the stain front, even though the stain may appear to be of a uniform color.

The quantitative aspects of gas adsorption in silica gel beds have been shown to be complex[13] and mathematical relations are best expressed graphically rather than analytically. We may state qualitatively that gas losses on the gel may be reduced and proportionally longer stains obtained by using higher flow rates. Furthermore, proportionally greater losses of a test gas would occur when it passes through a longer stain. This latter effect may be expressed as follows: For a fixed concentration, stain lengths will be proportional to the sample volume raised to a power less than one.

Some data showing the effects of flow rate and sample volume on the calibration of nitrogen dioxide indicator tubes were presented by Kinosian and Hubbard.[12] An empirical equation which fits their data reasonably well is as follows:

$$L = kC^a \left(\frac{V}{A}\right)^b \left(\frac{V}{At}\right)^c \qquad (4)$$

Here, k, a, b, and c are empirical constants. Constant k depends only upon the temperature. Constant a is not appreciably affected by sample volume and flow rate, and could be expected to lie between unity and $(1 - n)$ from equation (3). Constants b and c lie between 0 and 1. For the reference cited, best fit was obtained for values $a = 0.45$, $b = 0.41$, and $c = 0.84$.

It should be realized that equation (4) is applicable over only a limited range of flow rates, which is determined by the sorption rate of the test gas on the indicator gel. For higher flow rates, the stain length would exceed the tube length, and incomplete sorption would occur. Relations then degenerate to equation (1). For lower flow rates, the stained indicator gel attains the saturation level for the test concentration of gas, and the relations degenerate to equation (3).

Discussion

These relations are of importance when it is desired to deviate from the calibration volumes or flow rates in order to change the sensitivity of the tubes. A new calibration curve can be derived from the original one. If we use the general equation (4), and denote calibrated values by subscript 1, and test values obtained under different conditions by subscript 2, we may derive the following relation for a fixed stain length:

$$C_2 = C_1 \left[\frac{V_1}{V_2}\right]^{b/a} \left[\frac{A_2}{A_1}\right]^{(b+c)/a} \left[\frac{(V/t)_1}{(V/t)_2}\right]^{c/a} \qquad (5)$$

If it is desired to change the sensitivity of the tubes, ordinarily the flow rate and tube diameter remain constant and only the sample volume is changed. Thus the last two bracketted terms drop out. If the slope $(1/a)$ of the log-log calibration plot of concentration versus stain length is unity, it is reasonable to assume that b is also close to unity, and that the correction factor is the simple ratio of sample volumes. If the slope is steeper, the correction is less certain. In the case of the Kinosian and Hubbard data cited, the value of b/a is $0.41/0.45 = 0.91$, and the factor is somewhat less than the sample volume ratio. It is to be expected that the ultimate sensitivity attainable by increasing sample volume would be limited by weakening of the stain color and broadening of the stain front. Moreover, effects of interfering gases may be more serious.

The same quantitative considerations apply to corrections for random variations in tube diameters, which result in appreciable percentage variations in area in the narrow tubes. Calibrations for tubes in the B kit are based upon the use of a fixed volume of indicator gel in each tube, resulting in the filled length, S, varying inversely with cross sectional area. This length is measured on the calibration chart, and is subdivided into proportional segments representing the stain lengths for different concentrations. The mathematical basis for the corrections may be derived from equation (4), assuming a constant concentration, C, and constant flow rate V/t:

$$\frac{L_2}{L_1} = \left[\frac{S_2}{S_1}\right]^{(b+c)} \qquad (6)$$

Most of the calibrations indicate values of unity for the exponent $(b + c)$ in equation (6). The figures of tube sizes and diameter cited earlier in this report are for representative tubes taken at random. For example, tubes manufactured by company D vary in size dependent upon the particular gas or vapor concerned.

From the variations noted certain observations can be made. Variation in inside tube diameter becomes more significant with the smaller diameter tubes. For a given weight of gel, tube-to-tube inside diameter variations will result in inconsistent stain lengths. Device B attempts to compensate for any such inconsistency by providing a scale which relates the concentration to the ratio of the stain-length to total gel length. This is based upon equation (6) where the exponent is assumed to be unity. The manufacturer's calibration curves for tubes in the B kit show the L/S ratio to be approximately constant.

That the tube reaction is complex may be illustrated with the aid of Figure 2. The detector

tubes for NO_2, Cl_2 and Br_2 of manufacturer B are all identical and contain orthotolidine as the indicator on a silica gel matrix. Consider first the chlorine and bromine curves. If the gas reaction were limited to that with the indicator reagent it would be reasonable to expect that the curves would be nearly identical. The wide deviation implies that the sorption mechanism may be the major factor in the tube response. Bromine is more heavily adsorbed on silica gel than is chlorine, thus a shorter stain length would indicate a higher concentration. It is likely that the sequence of action in the tube is sorption by the gel with subsequent reaction of part of the sorbed gas with the indicator. As more data become available the nature of the process should become clearer.

Considerations in Tube Evaluation

With the exception of the tubes produced by manufacturers A and C, the calibrations for all other tubes are expressed in parts per million (ppm). Tubes in the A kit are calibrated in milligrams per liter (mg/liter) or milligrams per cubic meter (mg/m³). In the C kit a quantitative calibration is not used.

In an information sheet published by manufacturer A explaining the choice of the mg/liter calibration, the following statement is made: "...gas analysers based on a chemical principle of measurement always primarily indicate the weight of the gas in relation to the unit of volume. Thus a scale calibrated in mg/liter will always be found to produce absolutely correct measured values under all pressure conditions. Calibration scales in % vol. or in ppm are subject to errors when the measurements are made at greater height by which they are affected, and then require correction." A correction factor for temperature variation is also necessary. The relation between parts per million and mg/liter is given by:

$$\text{ppm} = \frac{\text{mg}}{\text{liter}} \times \frac{22{,}400}{\text{molecular wt.}} \times \frac{T \text{ abs.}}{273} \times \frac{760}{\text{pressure, mm Hg}}$$

A table of conversions for molecular weights from 1 to 300, by Fieldner, Katz, and Kinney[14] is available for convenience in making the calculation.

The instruction booklets provided for the several tubes of kit A include the conversion factor for the given gas at 760 mm Hg and 25°C. In several instances the conversion factor was found to be incorrect. For example, directions

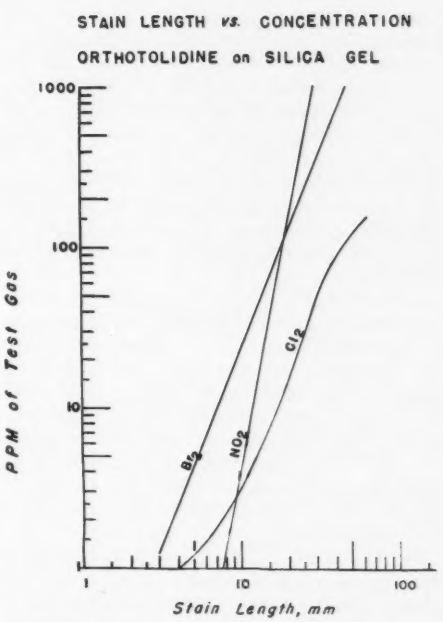

FIGURE 2. Stain length vs. concentration, orthotolidine on silica gel.

for the ammonia tube list as a conversion factor, 1 mg/liter = 1300 ppm, whereas the correct factor is 1438 at 760 mm Hg and 25°C. This is an error of about 10 per cent. The conversions for chlorine and hydrogen sulfide are incorrect by approximately 2 to 3 per cent. There can be little justification for providing an inaccurate conversion factor.

The volume of air sampled and the sampling rate for the determination of a given contaminant varies from manufacturer to manufacturer, and in some cases from detector to detector for a given manufacturer. For example, kit B, which utilizes a limiting flow orifice, has a total sampling time of 2, 2½, or 3 minutes for the 100 ml volume depending upon the tube. Kit A, has a sampling time of 5 seconds per 100 ml, but may require up to two liters for a given sample. This is equivalent to 20 squeezes of the bellows used for air aspiration. One detector of manufacturer D requires 50 squeezes of the aspirating bulb. A detector developed by the Naval Research Laboratory[15] for monoethanolamine and ammonia requires a 10 liter sample which is taken with an electrically operated pump for periods up to 40 minutes or longer. This variation in rate, volume, and length of time needed to take a single sample may lead to difficulties in interpre-

tation of final results. Sufficient discussion in the literature has been made regarding the difference between grab (or instantaneous) samples and long term integrated samples. It is not the purpose of this report to discuss the relative merits or the desirability of either technique. However, when assigning a certain risk potential to an industrial process based upon a given sample, the length of sample time must be considered. The effect of variation of flow rate upon accuracy of results is discussed elsewhere in this report.

Reliability of the air moving device must be considered in any evaluation of a gas detector. In a previous report[16] it was shown that variation in airflow, manufacturing errors, and deterioration of components with use could result in an altered airflow and hence, in a faulty estimate of the environmental concentration of the contaminant. It thus becomes important to assure consistent airflows from one sample to another. Frequent calibration of the air moving device is necessary.

Estimation of the gas concentration and interpretation of the potential hazard may be affected by the type of reaction; either color change or length of stain. A number of factors may affect the formation of the length of stain or make difficult its proper interpretation.

The particle size of the gel has an important effect on the ease of use of a tube. The line of demarcation at the stain front tends to become sharper as the size is decreased. The packing should be of sufficient uniformity and density to prevent channeling of flow. Where such channeling does occur, the downstream edge of the stain becomes highly irregular. Estimations in concentrations based upon the calibration for length at either of the two extreme stained edges have varied by significant factors.

Where the calibration scale is printed on the tube it is important that the zero point coincide with the edge of the gel packing. During many of the experimental runs an estimate of the zero point had to be made in deciding upon the interpretation of the stain length. For one type of hydrogen sulfide tube the deviation from zero was equivalent to 35 parts per million. Thus serious errors could result if the tubes were used to monitor environments by personnel not cognizant of the potential errors involved.

Judgment regarding the exact end-point of a stain may also be highly subjective. For example, the ammonia tube of kit B is colored pink. Upon exposure to NH_3 a fading to a cream-white or pale yellow occurs. At the demarcation line, the colors tend to blend with each other. Similarly, in device F, for carbon monoxide, a dark brown stain is formed in a yellow indicator gel. Near the interface, the intensity of the brown color is much less than that of the major portion of the stain. In both these examples, the interpretation of the end-point varied between observers.

Obviously, the problem of lighting is an important consideration here as well. In the case of tube F, greater uniformity between observers' interpretation was achieved by rewriting the manufacturer's directions for making the reading, and by suggesting to the readers that the tubes be read by transmitted rather than by reflected light. Where the gel and packing were such that light could be transmitted it was found that the end-point became easier to see when the light was placed behind the tube. Reflection and glare from the outer surface of the tube were minimized in this way. The effect on color of light on the estimation of tube response will be discussed with the color-change type of detectors.

The length-of-stain tubes of company C do not attempt to give an exact calibration. As an example, the following is taken from the manufacturer's directions for the detection of trichloroethylene.

Stain length	Interpretation	Approximate ppm
12–15 mm	Below M.A.C.	100
18–22 mm	At M.A.C.	200
26–30 mm	Above M.A.C.	400

Here, an uncertainty in three to four mm would be insignificant according to the manufacturer's calibration. It should be noted that the trichloroethylene detector of kit C has not been tested by this laboratory.

With the tubes in which a change in color, or change in the intensity of a color, is indicative of the concentration, other factors influencing concentration estimation occur. In general, there are two types of color changes to consider. Tubes for the detection of arsine, stibine, and mercury manufactured by company A, attempt to relate the concentrations to the first appearance of a color change. The number of squeezes needed to first produce a color at the leading edge of the indicator gel is proportional to the concentration. In arsine and stibine tubes of kit C, ten squeezes of the aspirating bulb are made and the concentration estimation is based on the following criteria as taken from the manufacturer's directions: "None, noticeable stain, definite stain."

In both tubes it is difficult to detect the exact moment of the color change. If there is any space at all between the leading edge of the gel and the pre-cleansing gel or the packing, the difference between a shadow and the first appear-

ance of a pale color is almost non-existent. With tube A a retrospective estimate of the concentration has to be made. This is done by noting the point at which the color change is intuitively felt to have occurred, then making an additional squeeze or two for confirmation. As one continues to test for these gases, however, the "feel" for the color change is intensified.

Where the intensity of a color is the determining factor, the ability to match shades will vary with the observer. For example, several series of tests were performed on the National Bureau of Standards type carbon monoxide detector. The reaction here is the reduction of a yellow complex silico-molybdate to molybdenum blue. The degree of color change from yellow to green to blue is a function of the CO concentration and the air volume sampled. In one series, three observers were asked to estimate concentrations based upon ten samples. Table I shows their interpretation of atmospheric concentration for a metered concentration of 100 ppm. The tubes used had a correction factor of two, hence a reading of 50 was equivalent to an estimate of 100 ppm. The data were analyzed by the analysis of variance. This provides essentially a uniform system for testing whether certain effects measured by the experiment, here observer differences and tube differences, can be considered to be sample values from a homogeneous population. The distribution of F for random samples drawn from normal populations is known. The probability of obtaining a value of F larger than any given value is known.[17] For the examples cited an F value greater than 3.55 would indicate that the probability of the differences between observers occurring as they did is less than 0.05. In fact, for the 0.01 probability level, the corresponding F value would be 6.01. Thus it would appear that the difference between observers in this one run was highly significant and could not be explained on the basis of chance.

A second illustration of observer variation was noted in tests performed on a hydrogen fluoride detector. Although this is not a tube type detector, it is readily available, and hence included in this report. The HF detector utilizes the change of color on a filter paper freshly treated with a liquid reagent as a means of estimating HF concentration. A drop of the test liquid placed on the paper forms a pale orange stain. Air contaminated with HF is drawn through the filter until a pale pink color appears. At the end point, the color is matched with a standard supplied by the manufacturer.

A panel of twelve observers was asked to interpret the colors from a series of HF samples

TABLE I

Observer Differences in Estimating Carbon Monoxide by NBS Detector Tubes (CO concentration 100 ppm*)

	Tube No.									
	1	2	3	4	5	6	7	8	9	10
Obs. 1	50	50	50	50	50	50	50	50	50	50
Obs. 2	75	75	75	50	75	100	75	100	75	75
Obs. 3	75	75	60	60	60	60	60	60	50	75

Analysis of Variance

Source	Degrees of freedom	Sum of squares	Mean squares	F value
Mean	1	121603		
Total	29	6247		
Observers	2	3781	1890	18.7—significant
Within Obs.	27	2466	140	1.4—not significant
Tubes	9	653		
Tubes × obs.	18	1813	101	

* Because of the tube scale factor all estimates are to be doubled. Thus a reading of 50 represents 100 ppm.

in the following manner. Air samples were drawn through the paper by means of the 50 ml bulb supplied as part of the kit. As the sample was taken, the color was to be noted independently after each aspiration. The observer would note at what point, if any, the color match was made. He was not to communicate the result until a total sample of 500 ml was taken. Even then, the observer's decision was noted in writing so that there was no communication between observers. After each run the panel left the room while the HF concentration was changed. The color matching was then repeated in the same manner.

Four runs were made in a 3½ hour period, using a single colorimetric preparation. Although the observers were told that a different HF concentration was prepared for each run, the same mixture was used for the entire series. The test concentration varied from 1.1 ppm at the beginning to 0.94 ppm at the end of the series of samples. Results of the estimates are given in Table II.

The observers were subsequently asked to take two pseudo-isochromatic color vision tests. Each one, with the exception of the two medical technicians who had left our employ showed normal color vision based on the Ishihara Test (A-O charts). The Keystone Industrial Visual Safety (IVS) Series, Test I, Color Discrimination

TABLE II

Observer Differences in Estimating Hydrogen Fluoride Concentration Based on Colorimetric Filter Paper Samplers. (average concentration 1.02 ppm)

Observer	HF concentration, read, ppm			
	Run 1	Run 2	Run 3	Run 4
Secretary	<0.5	<0.5	0	0
Junior Chemist	1	0.5	<0.5	<0.5
Chemist	1	0.5	0.5	1
Biochemist	1	>5	—	—
Chem-Technician	0	0	0.5	0
Med-Technician	1	>5	0.5	3
Med-Technician	>5	0	1	0.5
Chemist			0	0
Chemist			<0.5	0.5
Ind. Hyg. Engineer			<0.5	0.5
Ind. Hyg. Engineer			<0.5	1
Ind. Hyg. Engineer			<0.5	0

(Keystone View Company, Meadville, Pa.) was the second test used. The three subjects who tended to read low HF values were those who did poorest in the Keystone test.

The relation between what is considered normal color vision and the ability to distinguish subtle color changes is complex. The usual color screening tests may not adequately measure this ability. To this end, we are now in the midst of testing observers with the Inter-Society Color Aptitude Test.[18] This was developed for the purpose of detecting both those with above-average and below-average ability to distinguish small color differences both in the red-green and yellow-blue senses. We hope to present our findings in a subsequent report.

Interfering Gases and Vapors

Ideally, each type of detector tube should be specific for a separate gas. In practice, however, the reagents used are not unique, and thus one tube may detect several different gases. For example, only one type of tube in kit C is used for most halogenated hydrocarbons. In kit B the tubes for ethylene and carbon monoxide contain the same indicator. Tubes for NO_2, Cl_2, ClO_2, and Br_2 in kit B are identical. Where the gases are not found in the same environment, there is no difficulty with the analysis or interpretation of the data. Otherwise, the consequences that could result are illustrated by the following considerations.

A comparison of the kit B calibration charts for Cl_2 and NO_2 is shown in Figure 2. From the figure it can be seen that a test made for NO_2 might result in the estimate of a "safe" level, for example, 5 ppm. In reality, the atmosphere might contain 3.5 ppm of chlorine, a more dangerous situation. Conversely, a Cl_2 measurement (with the same figures) might lead to undue alarm, where in reality the color change was due wholly to NO_2.

It is apparent that in the calibration of tubes attention must be paid to the interferences. More important, however, is the interpretation of the results when interferences are present. The example cited referred to stain enhancement. In some cases an interference with color development occurs. Ayer and Saltzman reported on the interference of CO color development in the NBS tube with NO_2.[19] Although the NBS reagent is used in the kit B CO tube, a guard layer of reagent minimizes the interference at a concentration of 7 ppm NO_2. The type F detector which does not use the NBS reagent was not affected by a concentration of 7 ppm NO_2 in the presence of 25 ppm CO.

The last principle to consider in evaluating the relative merits of a tube is the consistency with which tubes from different production runs will respond. The principles of quality control have been thoroughly discussed in the statistics literature and are beyond the scope of this paper. Certain considerations should be mentioned here, however. A tube which does not respond according to the manufacturer's calibration curves is not necessarily worthless. If a preliminary examination showed that a series of tubes in a given lot indicated high by a given factor, there would be no need to disqualify that lot or type of tube, provided the factor was consistent from lot to lot. Statistical interpretation of calibration data with regard to variance in tube response becomes necessary. Furthermore, even a variation in this scale factor could be tolerated provided that limits to this variation were based on considerations of the toxicity involved, the direction in which the variation occurred (tubes reading too high or too low), and the use to which the tubes are to be put. Thus, from a rigid statistical viewpoint, calibration of the tubes by an industrial hygienist, might involve large numbers of tubes, perhaps 50, if the tolerance limits on tube performance were made rigorous. Furthermore, an attempt to use tubes so calibrated might necessitate taking 5 to 10 samples at each location in the field and having two or three observers reading the tubes. Such requirements are impractical to meet.

Thus, two alternatives are open to the user of such gas and vapor detecting devices. He may insist upon extensive quality control measures on the part of the manufacturer or his own laboratory, or he may use the tubes, fully

aware of any potential source of error involved in an interpretation of a hazard based upon one or two tube readings. For this alternative the results can be considered semi-quantitative at best.

Preparation of Known Concentrations

Techniques for preparing known concentrations of gas or vapor have been extensively described.[20] In the static system a known amount of gas or vapor is dispersed into an enclosure of known volume. As samples are withdrawn, air enters the enclosure to replace the sample volume. If instantaneous, perfect mixing occurs, then the concentration, C, remaining after a volum, V, has been withdrawn is:

$$C = C_0 e^{-V/V_0}$$

where C_0 is the initial concentration, V_0 the chamber volume, and e, the base of natural logarithms. Thus, after half the volume has been withdrawn the concentration remaining is reduced to 60.6% of the original. Setterlind[21] proposed the use of a series of five gallon or thirteen gallon glass bottles. For a series of five bottles, a withdrawal equal to twice the volume of a single bottle would leave a residual concentration of 98.8%.

The Industrial Hygiene staff of the Hanford Atomic operation[22] suggested the use of a carboy in which a plastic bag was attached to the replacement air tube. The replacement air would thus be prevented from diluting the sample. Where the test gas does not react with the bag, and where the wall effect is negligible, or exactly known, this would be a convenient method of calibration.

The dynamic method, the one used by our laboratory, consists in diluting a metered stream of known concentration with a metered stream of clean air to produce the desired mixture. The only limitation on sampling volume or rate is the practical one caused by available equipment. Accurate knowledge of the stock concentration and accurate measurement of gas flows in the two streams is necessary. Where low concentrations are desired, multiple dilutions may be necessary. Commercially available rotameters usually do not permit the measurement of less than a few ml per minute. Thus, if 2 ml of the gas can be metered accurately, and if the source of gas is from a cylinder (theoretically, 100% pure) a diluting volume of 20 liters per minute would be needed to produce a concentration of 100 ppm. Obviously for lower concentrations impractical diluting volumes are necessary.

Saltzman[23] described a flow meter capable of measuring flows as low as 0.01 ml per minute. Gas is metered through a tightly packed asbestos plug in a three-way, T-shaped capillary stopcock. The plug comprises acid washed, medium fiber asbestos (of the type used for Gooch crucibles) packed in one leg of the one mm bore of the stopcock. See Figure 3. Pressure on the plug face is maintained constant by discharging the gas stream through the other leg of the bore through a water bubbler with known head. Gas flow is varied by changing the head on the discharge tube. Calibration is carried out by timing the rate of flow of a water droplet in a 1.0 or 0.1 ml graduated volumetric pipette attached to the gas delivery end.

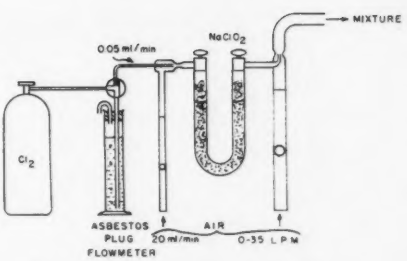

FIGURE 3. Apparatus for generating chlorine dioxide—air mixtures showing asbestos plug flowmeter.

Nitrogen dioxide and other gases with low liquefaction temperatures cannot be used because of the possibility of fouling the plug. Back pressure on the flow meter from the system must be considered in making calculations. Experimental work with ammonia showed that this gas was not amenable to metering, apparently due to reaction with water vapor in the flow system.

Rotameters as supplied are calibrated for air flow at atmospheric pressure and 70°F. Variations in flow with temperature and pressure or with gases of different densities and viscosities are compensated for by application of correction equations. One rotameter manufacturer supplies correction curves for gases of different densities. Calibrations of the rotameters against a spirometer, calibrated wet test meter, and for low flow rates, against the "soap bubble" meter[16] showed that the manufacturer's air flow calibration was not always exact at all rates. Flow curves based on the supplied correction equations especially for gases other than air were not always exact. It is recommended that all rotameters be calibrated under conditions of use.

Provision for simultaneous sampling for chemical analysis should be made to control experi-

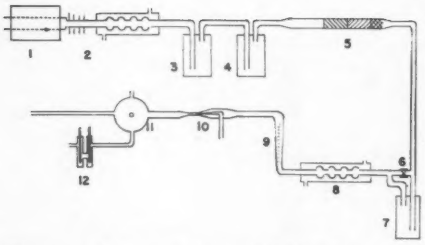

FIGURE 4. Apparatus for establishing known concentrations; dynamic systems.

mental errors in metering. Variations in flow either of the test gas or the dilution air may occur during an experimental run. Further, as described elsewhere in this report, tank gases are not always pure or at the concentrations nominally indicated. In this laboratory we use a 500 ml round bottom flask with a 35/20 standard ground joint as the sampling chamber. Sections of two millimeter capillary tubing, about 1½ inches long, are sealed 90° apart around the periphery of the flask. These serve as sampling ports. The discharge tube is an 18-inch length of 10 mm glass tubing at the base of the flask. A sketch of the sampling chamber is shown as part of the dilution system in Figure 4.

The low concentrations of gases involved in the final calibration step require that the diluting air be rendered contaminant free. The system recommended by us is shown in Figure 4. Air from the laboratory compressed air line is passed through a filter-regulator and then through a 2-inch mullite tube in a furnace (1), the interior temperature of which is kept at 1250°F. The tube is loosely packed with copper turnings. At this temperature combustion of many of the organic impurities in the air stream occurs. At a flow of 60 liters per minute, the exit temperature was found to be 800°F. A cooling train comprising a finned brass tube and a water-cooled condenser (2) are used to reduce the temperature to about 120°F. The air is then passed through sulfuric acid-dichromate cleaning solution, an acid trap, ascarite (sodium hydroxide on asbestos), activated charcoal and a glass wool filter (3–5). A bypass (6) is provided so that a portion of the airstream may be passed through

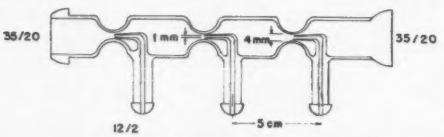

FIGURE 5. Gas mixing apparatus.

a humidifying bubbler (7). An Allihn condenser (8) which is fed from a constant-temperature bath is used to regulate air temperature. The purified dilution air then passes through a rotameter (9) and into a mixing tube (10). The gas-mixing tube is shown in detail in Figure 5. The sampling chamber (11) is shown with a wet and dry bulb thermometer assembly (12) for determination of relative humidity.

The gas-mixing device, Figure 5, is designed to produce high turbulence at the point of test gas introduction to assure complete mixing. The narrow bore was chosen for the gas inlet in order to keep dead space to a minimum. Rotameters with standard ball joints are commercially available as stock items and can be attached directly to the inlet. The use of all glass connections is to eliminate losses by adsorption on or reaction with rubber or plastic tubing.

Sources of Test Gases

Where available, tanks or lecture bottles of compressed gases were purchased from commercial sources. In all cases the manufacturers' values were taken as nominal and the tank concentrations were checked for any deviation from the labeled concentration. With nitrogen dioxide, for example, there were serious discrepancies between the analyzed and nominal values of the tank mixture used. It appeared as though no allowances were made by the supplier for large deviations from the ideal gas law. Tank gas of nominal 1% NO_2 in air and 1% NO_2 in nitrogen were used. If it is assumed that one atmosphere of NO_2 was introduced into an evacuated tank, and the latter was pressurized with air or nitrogen, the actual concentration would be found to be 1.7%. This is due to the partial reversible dimerization according to:

$$2\ NO_2 \rightleftarrows N_2O_4$$

The extent of this effect, shown in Figure 6, is calculated from data by Verhoek and Daniels.[24]

Reference has been made to errors caused by the liquefaction in the tank. The boiling point of NO_2 at one atmosphere is 21.3°C. Thus, in the filling operation, it is possible for NO_2 to condense. The percentage composition of a gas mixture in a tank containing liquid NO_2 is not constant, but is equal to the vapor pressure of NO_2 (corrected to ideal conditions) at the tank temperature divided by the total pressure of gas in the tank. Such a calculation is complicated because the high tank pressure affects the vapor pressure of liquid NO_2.

Detailed descriptions of the preparation and analysis of known mixtures of gases referred to

in this report have been given elsewhere.[25] Brief mention will be made of some methods of interest. Chlorine dioxide was prepared by passing a known chlorine-air mixture through sodium chlorite which completely converted the chlorine to ClO_2. The preliminary dilution was necessary because concentrations of ClO_2 in air greater than 5% are explosive. Simultaneous analyses of the low levels were made with neutral and acid iodide solutions. Chlorine liberated the same amount of iodine from either reagent, whereas ClO_2 liberated five times as much from the acid as from the neutral reagent.

Arsine and stibine were generated electrolytically, in the apparatus shown in Figure 7. This comprised a two-necked 250 ml flask with standard taper points through one of which a pair of platinum wire electrodes (5 and 6) with Teflon insulation (7) were inserted. The second neck contained an air inlet (1) terminating in a submerged fritted glass tube (4) and an air outlet (2). The electrolyte (3) was sodium arsenite in sulfuric acid for the arsine; antimony in tartaric acid for the stibine.

For carbon monoxide, 0.1% CO in air tank gas was used. Analysis was made by passing the gas through an absorbing train containing Ascarite, Hopcalite, and Ascarite to remove carbon dioxide initially present, to oxidize CO to CO_2 catalytically, and to absorb the resultant CO_2. This procedure was found to be more convenient than the iodine pentoxide method.

Test Results

Results of tests to June 1960 are presented in Table III. It should be emphasized that these results reflect only the response of the tubes tested and are not necessarily indicative of the performance of other tubes manufactured by the various companies. As indicated previously in this report, production changes may result in changes in tube characteristic. The reader is cautioned to calibrate his own detector tubes for greater accuracy.

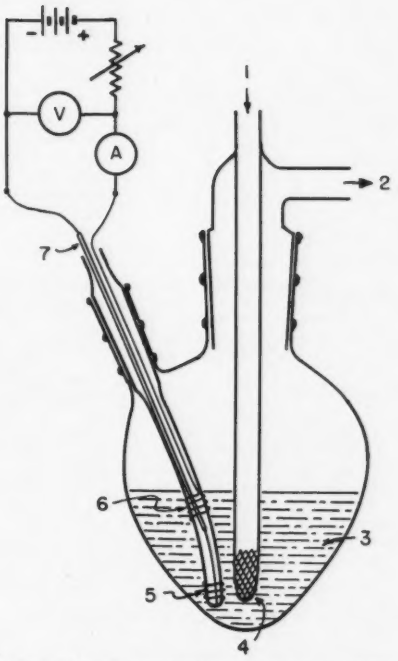

FIGURE 7. Electrolytic generator assembly.

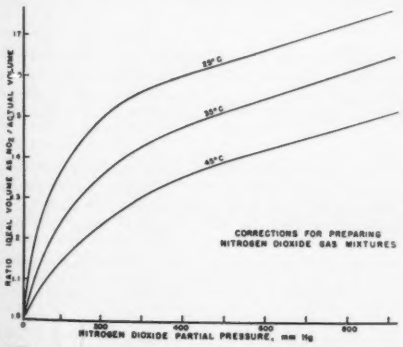
RATIO IDEAL VOLUME AS NO_2 / ACTUAL VOLUME
25° C
35° C
45° C
CORRECTIONS FOR PREPARING NITROGEN DIOXIDE GAS MIXTURES
NITROGEN DIOXIDE PARTIAL PRESSURE, mm Hg

FIGURE 6. Corrections for preparing NO_2 gas mixtures.

For all gases listed, test runs were made at 0.1 TLV, the TLV and 10 TLV concentrations. For the carbon monoxide series, tests were made at 2.5, 25, and 230 ppm. The "standard interference" mixture referred to in the table comprised: CO_2—30,000 ppm; CO—50 ppm; CH_4—200 ppm; H_2—35,000 ppm; CCl_2F_2—1,000 ppm.

Summary and Conclusions

Theoretical and practical principles for the calibration of direct reading colorimetric gas detecting devices have been presented. A mathematical approach to calibration has been given. It is hoped that this approach can be used by others in the industrial hygiene field as a basis for future work. Successful calibration and analytic techniques have been presented. Test results for certain tubes have been listed and discussed. It is reemphasized that the tests were basically for the needs of this laboratory and were not exhaustive or necessarily representative of the present state of tubes commercially avail-

TABLE III
Results of Tests on Specific Gases

Gas (TLV)	Tube	Response
Arsine (0.05 ppm)	A	Indication is by abrupt color change from yellow to pale gray, difficult to see. Tubes read high (safe side) by factor of 2.5. One run at 0.072 ppm resulted in estimates ranging from 0.31 to 1.1 ppm with mean of 0.45 ppm (factor = 6.25). To users inexperienced with this tube errors on unsafe side may occur.
	B	High range phosphine tube responded to arsine. Length-of-stain tube, color change from light blue to brown, easy to see. Potentially capable of calibration and use as arsine detector.
	C	Arsine tube unavailable at time of test, but same tube used for stibine.
Carbon Monoxide (100 ppm)	A	Length-of-stain tube, color change from white to blue difficult to evaluate as two separate shades developed. Result of estimates erratic at concentrations ranging from 2.5 to 230 ppm.
	B	Color-change tube, yellow to green to blue. See text regarding observer color judgment. Gave good results at 25 and 230 ppm, showed ability to detect CO at 2.5 ppm. Not affected by 200 ppm CH_4 or 7 ppm NO_2. Use of temperature correction chart supplied led to some error.
	C	Color-change tube, from yellow to green to blue, same as D. This tube was not tested.
	D	National Bureau of Standards color-change tube. Change from yellow to green to blue. See text regarding observers' color judgment. Response at 25 and 230 ppm good. Ability to estimate concentration of 2.5 ppm. At 100 ppm some differences between observers' interpretation of colors noted (see text). No interference with 200 ppm CH_4. Serious interference with 7 ppm NO_2.
	E	National Bureau of Standards color-change tube, same as D.
	F	Length-of-stain tube, color change from yellow to dark brown, very easy to see. Good response at 2.5, 25, and 230 ppm. No interference with 200 ppm CH_4 or 7 ppm NO_2.
NO_2 (5 ppm)	A	Length-of-stain tube, color change from white to orange, read with some difficulty. Read high by a factor of 2 to 4 at 5 and 77 ppm. Some response shown at 0.1 ppm. Error here is on safe side. No interference from standard mixture.
	B	Length-of-stain tube, color change from white to brown, fairly easy to read. Very erratic results from tubes on hand. At 4.9 ppm, of 17 tubes taken at random, eight were poorly packed; remainder estimated range 0.5 to 10 ppm with mean of 2.4 ppm (unsafe error). At 77 ppm, response ranged from 30 to 500 ppm. Manufacturer indicates improved tube now available. Also see text regarding reaction of this tube with chlorine and chlorine dioxide.
Stibine (0.1 ppm)	A	Color-change tube, abrupt change from yellow to gray. Immediate color change hard to see. Uses arsine tube, no stibine calibration supplied. At 0.08 ppm mean response was 0.04 ppm with range of 0.02 to 0.06 based on arsine calibration scale. At 0.64 ppm mean estimate was 1.1 ppm. Tube responded at 0.006 ppm. Negligible effect with standard interferences. Incorrect instructions were packed with tubes used.
	B	No response with phosphine tube.
	C	Color-change tube, abrupt change from yellow to gray. Ease in seeing color depends upon relative concentration. Tube performed as claimed by manufacturer. Not affected by standard interferences.
Phosgene (1 ppm)	A	Length-of-stain tube, color change from blue to green. Leading edge of stain sharp. Very easy to see. Tube response excellent, with scale factor of approximately 2 (safe side). Bleaching of color noted after 4 to 8 hours. Tube could then be reused once. Questionable effect from standard interferences, causing tube to respond closer to manufacturer's rating.
Chlorine (1 ppm)	A	Length-of-stain tube, color change from white to orange, fairly easy to read. Correction factors of two batches at 1.1 ppm were 4 and 2.5. Analysis of variance showed significant differences between lots. Tests run six months later showed scale factors of 1.8. At 11 ppm, scale factor was approximately 2. All errors were on safe side. Many tubes in early lots were poorly packed.
	B	Length-of-stain tube, color change from white to brown, easy to read. Mixed response; one batch gave no readings; one batch showed mean concentration of 1.2 ppm (range 1–1.75 ppm) when exposed to 1.1 ppm.
Chlorine Dioxide (1 ppm)	A	Length-of-stain tube, same as for chlorine tube A. At 1 ppm, estimations ranged from 1.7 to 2.5 ppm. At 0.14 ppm estimates ranged from 0.25 to 0.29 ppm. Scale factor of 2 appeared to be consistent.

TABLE III—(*Cont.*)

Gas (TLV)	Tube	Respone
Chlorine Dioxide (1 ppm)	B	Length-of-stain tube same as for type B chlorine and NO_2, but different calibration curve. At 1 ppm estimates ranged from 1.75 to 2.0 ppm. At 0.14 ppm estimates ranged from 0.1 to 0.2 ppm. Scale factor and response appeared to be consistent.
Mercury Vapor (0.5 mg/m³)	A	No response at TLV values. Tube indicated presence of Hg vapor when placed in flask containing the metal.

able. The results reported do not imply endorsement or disapprobation of a manufacturer or device by the Public Health Service.

The present state of gas detecting tubes is that they are, in general, semi-quantitative devices quite useful in screening or preliminary survey work. Use of these tubes and the interpretation of results obtained by such use should be made by a qualified industrial hygienist, aware of the toxicity of contaminants involved and of the tube performance. Where facilities and trained personnel are available calibrations of the tubes and auxiliary equipment should be made before their use.

Acknowledgments

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The Effects of Continuous Exposure of Animals to Ethanolamine Vapor

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Introduction

MONOETHANOLAMINE (MEA) is a stable aliphatic amine with a wide range of industrial uses. Its affinity for acid gases and its high absorption efficiency make it a useful agent for removing carbon dioxide and similar gases from exhausts of industrial wastes and from gaseous and petroleum products. Its application to air purification processes raises the question of possible deleterious effects on man in the event that some MEA is carried into the air stream during the passage of air through scrubbers containing aqueous solutions of monoethanolamine.

Biologically, monoethanolamine is a normal intermediate in animal metabolism having a role in the formation of phospholipids and choline.[1] A certain amount of free MEA is excreted in the urine. Most of the work on the metabolism of MEA has been done with rats. Some species, notably guinea pigs, chicks, and ducklings, differ from rats in that they are unable to form choline from MEA.

Hinglais[2] found, when comparing the irritant and necrotic action of mono-, di-, and triethanolamine and their hydrochlorides, that the effects were not related to pH (9 to 11 for the free bases). He reported that monoethanolamine was the most potent irritant of the three and had considerable necrotic action on the rabbit ear. Manufacturers of ethanolamines recognize that these compounds are skin irritants and that prolonged or repeated contact with the skin should be avoided. They recommend that proper ventilation and adequate protective clothing be employed and that undiluted material coming in contact with the skin or eyes should be flushed immediately with water.[3]

The pharmacological effects of this compound have been demonstrated in studies on nembutalized dogs at the Mellon Institute.[4] Depending on the dosage used, MEA produced either central nervous system stimulation (with low doses) or central nervous system depression (with lethal doses).

Ethanolamine has a low oral toxicity, but can be dangerous if swallowed in large quantity and may also cause considerable local injury. The quantitative subacute oral toxicity of MEA administered in food to rats for 90 days has been reported by Smyth *et al.*[5] They found that rats were not affected by 0.5% MEA in their diet (320 mg/kg/day).

Treon *et al.*[6] have investigated the effects of inhalation of high concentrations of MEA for short periods of time. Their studies were mainly a repetitive type of exposure although they compared continuous exposures of a few days with similar exposures that were intermittent in nature. They ascertained that there was no difference in mortality resulting from the physical state of the MEA in the air, whether it occurred as a mist or as a vapor.

The toxicity of MEA was investigated in our experiments by determining the effects in animals from continuous exposure to various concentrations of its vapor. Additional studies were conducted for the purpose of determining the maximum concentration to which animals could be continuously exposed without producing harmful effects.

Investigational Procedures

Properties

Monoethanolamine ($HOCH_2CH_2NH_2$) is a very hygroscopic organic base, and has about the same alkalinity as ammonia. No thermal decomposition of the amine occurs at temperatures as high as 184°C.[7] Chemical assay of the MEA used in our experiments showed the following proportion of components:

Diethanolamine	
Triethanolamine	0.2%
Water	0.35%
Ammonia	<0.1%
Monoethanolamine	99.3%

Table I lists some physical properties of MEA and of 30% (w/w) aqueous MEA.

TABLE I
Physical Properties

	Monoethanolamine	30% Aqueous monoethanolamine (w/w)
Mol. wt.	61.08	—
B.P.	170.5°C	103–104°C
State	Clear, viscous, liquid	Clear, liquid
Specific gravity	1.0179 @ 20°C/20°C	—
Solubility	Infinitely soluble in H_2O and alcohol	—
pH	—	12.75
Vapor pressure	0.4 mm Hg @ 20°C	28 mm Hg @ 30°C

Dispersion

Volatilization of MEA was effected from a 30% aqueous solution by means of the apparatus shown in Figure 1. The solution was dropped at a constant rate from a Mariotte bulb reservoir onto gauze covering the internal perforated glass column suspended in one end of the large U-tube. Air, passed through and over the gauze, carried the vapors up to a bowl where they mixed with the affluent air of a dynamic gassing chember.[8] To facilitate uniform vaporization constant temperature was maintained in the area of evaporation by means of a heating jacket around the outside of the U-tube. The non-volatilized excess was constantly drained from the bottom of the U-tube.

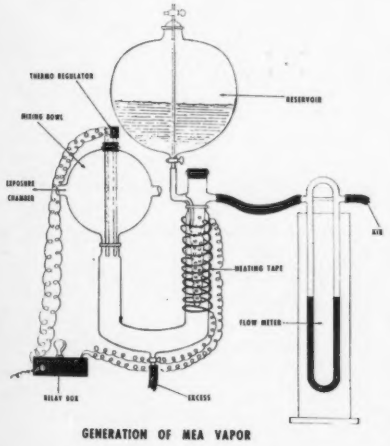

FIGURE 1.

Chamber Analyses

Atmospheric concentrations of MEA and of CO_2 were determined by chemical analysis of air samples collected from the room and from each exposure and control chamber. Vigreaux-type bubblers containing water were used to collect air samples for MEA analyses. Aliquots from these bubblers were analyzed for MEA by titration of the base with normal HCl or by a colorimetric method based on the determination of formaldehyde.[9] Analytical values based on these methods compared favorably with those determined by the more laborious but equally accurate ninhydrin method.[10] Carbon dioxide was determined by a standard acidimetric method[11] from samples collected in bubblers containing standardized (0.1*N*) sodium hydroxide. In addition, daily recordings were made of the relative humidity and temperature.

Experimental Animals

Experimental animals used were mature, male beagle hounds immunized against distemper, infectious hepatitis and rabies; male guinea pigs, six weeks old and weighing about 350 grams; female rats, eight weeks old and weighing about 160 grams in exposures to high and intermediate concentrations and male and female rats, 4–5 weeks old and weighing about 80 grams in exposures to low concentrations. Rodents were from the CW Laboratories colonies; rats from this colony are derived CFW animals while guinea pigs are derived Hartley strain animals.

Three unrestrained dogs were housed together in each 700-liter exposure chamber, fed cooked meat once a day, and given water approximately every two hours. Food and water not consumed after 15 minutes were removed to avoid contamination by the MEA vapor. Group cages in 400-liter chambers were used to house the rodents. Water and standard diets of pellets were available to the rodents *ad libitum*, plus fresh leafy greens for the guinea pigs. Water and food were replaced by fresh supplies at 4-hour intervals. Control animals were kept under identical conditions except for the presence of MEA vapor.

Clinical and Pathologic Procedures

The toxic action of MEA on animals was evaluated by observing behavioral changes, clinical signs, and mortality, and by making macroscopic and microscopic examinations of organs and tissues. Observations of behavior, appetite, elimination, weight, and clinical signs were recorded daily. Blood samples were taken from the radial vein of each dog and each week a physical examination was made which checked skin, eyes, ears, pulse, and heart and lung sounds. Rodents were sacrificed each week for necropsy at which time intracardiac blood samples were taken.

Blood samples were examined for changes in

prothrombin time (diluted and undiluted),[12] total protein, albumin and globulin,[13] inorganic phosphorus,[14] sulfobromophthalein retention,[15] serum phospholipids,[16] non-protein nitrogen,[17] calcium,[18] chloride,[19] sodium and potassium,[20] carbon dioxide,[21] hemoglobin, hematocrit,[22] and blood cell counts.

Exposure Schedule

The exposures were continuous, 24 hours/day, 7 days/week, except for a short period of about 15 minutes each day during which time the cages and chambers were cleaned.

Table II presents an outline of the various exposures showing the species, duration and the average atmospheric concentration of MEA.

TABLE II

Exposure Sequences to MEA Vapor

Species	Sex	Number exposed	Length of exposure	Average concentration of MEA	
			days	ppm	mg/l
Dog	M	3	30	102	0.250
Guinea pig	M	22	24	75	.184
Rat	F	45	30	66	.162
Dog	M	3	90	26	.064
Dog	M	3	90	12	.029
Guinea pig	M	30	90	15	.037
Rat	F	45	90	12	.029
Dog	M	3	60	6	.015
Rat	F	20	40	5	.012
Rat	M	20	40	5	.012

Odor Tests on Man

The odor detection level of monoethanolamine vapor for man was investigated using the Fair-Wells osmoscope. Each individual was asked to describe the odor at the first level at which it could be detected. The median detectable concentration was computed by the time-per cent effect method of Litchfield.[23]

Results

The temperature, relative humidity and CO_2 concentration of air in the exposure chambers as well as the control chambers are shown in Table III. There was no attempt to differentiate between free MEA and MEA combined with CO_2. The CO_2 content in the control chambers was equal to or slightly higher than the CO_2 content in the exposure chambers. In general, the higher the MEA concentration, the lower the CO_2 content found in the exposure chambers. The excess MEA that was not volatilized by the dispersion apparatus showed a higher viscosity than the original solution and was brown in color.

For ease of presentation the results have been divided into sections on toxic signs, pathologic observations, skin changes, blood studies, and odor detection tests. The first two sections are further subdivided into groups according to the level of MEA vapor to which the animals were exposed. One includes dogs, rats, and guinea pigs exposed to high concentrations of 102, 66, and 75 ppm MEA, respectively. Another subdivision includes similar groups of animals exposed to intermediate concentrations of 12–26 ppm MEA.

TABLE III

Summary of Experimental Conditions During Monoethanolamine Exposures

Species	MEA concentrations ppm ± SD	Temperatures (°C)			Carbon dioxide concentrations (ppm)			Relative humidity (%)		
		Exposure chamber	Control chamber	Room	Exposure chamber	Control chamber	Room	Exposure chamber	Control chamber	Room
Rat	4.8 ± 0.4	27	27	25	1457	1577	365	70	73	59
	12.1 ± 2.9	27	27	25	1043	1038	290	68	65	61
	66.3 ± 28.3	28	—	23	868	—	284	59	—	73
Guinea pig	15.0 ± 4.0	26	24	23	1245	1449	296	79	78	74
	75.2 ± 6.5	27	—	23	559	—	437	65	—	63
Dog	6.0 ± 3.4	27	26	24	1607	2217	292	68	57	50
	12.0 ± 3.4	27	26	25	1200	1803	308	65	74	64
	26.5 ± 7.6	28	27	25	861	1770	315	63	75	65
	102.0 ± 32	29	26	23	1046	2716	359	—	—	—

The third subdivision includes groups of dogs and rats exposed to low concentrations of 5 and 6 ppm MEA.

Toxic Signs

A. Exposures to high concentrations of MEA vapor. Maximal toxic effects and deaths were seen in animals exposed to high concentrations of MEA (66–102 ppm). Dogs tolerated a much higher vapor concentration of MEA and for a longer period of time without dying than did rodents. Two out of three dogs survived 30 days of exposure, the other dying after 25 days, while 83% of the rats and 75% of the guinea pigs died after 28 and 24 days of exposure, respectively. The rats began dying after 14 days of exposure and the guinea pigs after 10 days. Using the method of Litchfield[23] for calculating time-effect responses, the survival times for one-half of the exposed rats and guinea pigs were 19–20 days with a common slope function of 1.20.

(a) DOGS: The effects noted in three dogs (Nos. 177, 179, 180) exposed to 102 ppm MEA for 30 days were quite marked. At this concentration MEA condensed on all surfaces causing the pelt to become wet, matted, and greasy. On the first day of exposure dogs showed immediate discomfort by an uneasy demeanor, scratching the chamber door, looking for rescue, panting, muzzle licking, and vigorous head-shaking. Within a few hours they were salivating and vomiting. They became progressively more restless and quarrelsome, fighting between periods of quiet. Within 24 hours of initiation of exposure all were depressed. Attempts to attract their attention by knocking on the chamber door (normally enough for enthusiastic response) produced no response other than eye shifting. Their apathy and depression progressed to lethargy in 48 hours. Their appetites were immediately affected, improved somewhat after three days, but were not good any time during exposure. The feces began to soften on the sixth day and this condition progressed to diarrhea by the eighth day, but improved about the eighteenth day.

Two dogs (Nos. 179, 180) developed moist rales by the middle of the second week. This was associated with low grade fever (103–104°F), which ran a course of about two weeks. On several occasions dog No. 179 seemed moribund but recovered each time until the 25th day, when death occurred. No apparent respiratory involvement was observed in the third dog (No. 177). Leg muscle tremors were noted in the dogs after about seventy hours; these increased variably in severity during the exposure. The eyes appeared normal.

Head-shaking by the animals at the beginning of the exposure contributed to hematomas at the base of the ears and edema at the ear margins. Though head-shaking stopped after the fourth day, most of the ear margin tissue was necrotic at the end of the experiments. The skin around the scrotum and sternum became raw and sensitive about the fourth day of exposure. At these and other points, this condition gradually progressed to ulceration; by the ninth day the ulcers were covered by a thick black eschar. Soothing ointment (Vio-stringent, Pittman-Moore Co.) did not seem to relieve the irritation. The areas of ulceration were (1) body-to-floor contact regions (sternum, scrotum, foot pads), (2) poorly aerated areas (major skin folds over joints, between toes, and under ears), and (3) skin tension areas (around eyes, nose). The feet of the two heavier dogs (Nos. 179 and 180) became so sensitive that they would not voluntarily walk.

(b) RODENTS: Rats and guinea pigs exposed to high MEA concentrations (66 and 75 ppm, respectively) exhibited signs similar to those noted in dogs exposed to 102 ppm. Their coats quickly became wet and greasy to touch, and the animals were restless, irritable, and obviously uncomfortable, with greater breathing effort and high water consumption. After initially increased activity they became progressively less alert and appeared dejected, apathetic, and depressed. They tended to huddle in corners without moving, and it was difficult to determine whether they were alive. Once they were stimulated, their agility did not seem impaired, but they returned quickly to their apathetic state when left undisturbed. Death was sudden, with no apparent signs other than a few gasps. The hair became thin on rats during the experiment, particularly about the face and back. Guinea pigs did not appear to lose hair, but on necropsy, large tufts could easily be pulled from the skin. The ear margins of guinea pigs were noted to darken in about a week with formation of eschars within two weeks. This was not noted in rats, although other skin lesions in rodents were similar. Ground contact points (feet, nose, lips, chin) and skin points of tension (around extensor surfaces of large joints) showed dark black eschars before two weeks; these became progressively thicker with time and could be pulled free, revealing ulcerated skin beneath.

B. Animal exposures to intermediate concentrations of MEA vapor (12–26 ppm). Exposure for 90 days to concentrations of 12–26 ppm MEA did not cause fatalities in any species and signs

were generally similar to, but not so severe as, those of animals at the high concentrations.

(a) DOGS: Dogs exposed to an MEA concentration of 26 ppm showed immediate signs of restlessness and discomfort indicated by nose-pawing, muzzle-licking, and shallow, rapid respiration. Throughout the experiment they were more irritable than controls and after a few days' exposure they were less alert and thereafter bordered variably on lethargy. Slight tremors of rear leg muscles were noted. Within two days of exposure their coats became wet, greasy, matted, and were an obvious source of discomfort. Skin at floor contact points on chest and scrotum became irritated and in less than a week showed small ulcers. Ointment and astringent applications gave relief, but hair sparseness, small black eschars, and numerous small cutaneous pits noted at necropsy indicated continued irritation.

Dogs exposed to 12 ppm of MEA for 90 days did not show immediate behavioral changes. The weight changes were not significant nor did the physical examinations reveal any changes. After several days, the hair of the animals became dingy, and the skin was irritated, as revealed by attempts to rub against chamber walls. When the scrotal and chest skin became irritated it was treated with soothing ointment. This relieved the condition and the skin showed no further signs of irritation. Concurrently, lethargy or depression appeared and lasted about three weeks before the dogs' behavior returned to normal.

(b) RODENTS: Rats and guinea pigs exposed to 12–15 ppm of MEA became less active than the controls after about three days, and showed definite lethargy after about ten days, which lasted throughout the balance of the exposure. Early in the exposure, the coats of the rats became thin about the back and tailbase. The hair reappeared after a few weeks but then was lost again. Rodents showed about a 10% reduction in weight gain and about a 40% increase in water consumption.

C. Animal exposures to low concentrations of MEA (5–6 ppm). Mature, male beagle dogs (formerly the 90-day chamber controls) and groups of young immature (4–5 weeks old) male and female rats were exposed to 6 and 5 ppm of MEA for 60 and 40 days, respectively. Behavioral changes in animals occurred only after 2–3 weeks of exposure at these low concentration levels.

(a) DOGS: In dogs a slight decrease in alertness and activity was noted. Two of the three exposed dogs showed a slight weight loss. No changes from normal were observed in pulse, temperature, and heart and lung sounds. During the course of the exposure the dogs' coats gradually became dingy yellow and slightly greasy, a condition that apparently caused some discomfort and skin irritation. The skin at chest-floor contact areas lost hair, and the scrotum became bare and spotted with small scattered black eschars.

(b) RODENTS: All rats exposed at 5 ppm showed pelt discoloration after 12 days and transitory hair loss on the head and back after three weeks. This was more pronounced in the females than in males. Some slowness in movement developed after three weeks exposure. The rate of growth of these young rats was not different from that of their controls.

Pathologic Observations

A. Animals exposed to high concentrations (66–102 ppm MEA).

(a) DOGS: At necropsy the pancreases of the dogs appeared shrunken and gray, with a few reddish spots on their surfaces. Other than skin lesions, the two animals that survived the experiment did not show outstanding gross changes.

On incision, excess fluid ran from the ventricles of the brain of one of the animals. The lungs of both had several raised, reddened areas approximately one centimeter in diameter, and the mucosa covering the turbinates was pale. The alimentary tract was empty except for varicolored mucus; toward the lower end of the small intestine the wall became thinner; the colon was distended with gas, and its wall was paper-thin.

Histopathologic changes noted in the two surviving dogs were more pronounced than in the one that died during exposure. Central veins in the liver were congested, and over large areas the cytoplasm of each parenchymal cell contained a half-dozen small vacuoles. In some lobules the parenchymal cells were swollen slightly, and the cytoplasm was somewhat cloudy. The Kupffer cells contained more brown pigment than is usual. In the spleen the Malpighian corpuscles were depleted of lymphocytes, and in the red pulp there were fewer red cells than usually seen. Macrophages in the spleen contained more brown pigment than usually is noted. The kidney tubules contained increased numbers of hyaline pink granules, indicating some increase in protein excretion, and mild cloudy swelling was noted in epithelium of the convoluted tubules. The turbinate mucosa showed slight erosion in some areas and plasma cell infiltration in others. The lungs showed general congestion, small focal areas of alveolar

hemorrhage, and air passages containing blood. Spermatogenesis was repressed.

The dog that died during exposure showed scattered areas of moderate lobular pneumonitis with irregular areas of bronchitis and bronchiolitis. Splenic corpuscles were depleted of lymphocytes, and there were fewer red cells than usual in the red pulp. Sperm formation in the testes was suppressed. Other tissues examined, including pancreas, intestine, and brain, appeared normal.

(b) RODENTS: At necropsy, no gross changes of internal organs were noted. However, in a large percentage of animals that died during exposure the large intestine was distended with dry, packed fecal pellets. Many of these animals were presented for necropsy in various stages of post mortem decomposition.

Histologic study revealed, in a majority of exposed guinea pigs, general disseminated fatty metamorphosis of the liver and a similar but less severe perilobular condition in a smaller percentage of exposed rats. Areas of slight cloudy swelling were not uncommon in liver cord cells, a condition noted also in the tubular epithelium of kidney. Lymphocytic infiltration of lungs in guinea pigs was a common finding, more frequent in exposed animals than in controls. The condition varied from a few small foci to numerous large foci with confluent borders involving entire lobules, most of the lobes, or the greater part of the lungs. In the rat, increased disseminated collections of interstitial lymphoid tissue were noted, as well as some areas of mild patchy pneumonitis. The bone marrow appeared essentially normal, but in some guinea pigs normoblasts and granulocytes appeared to be more numerous than usual. Spermatogenesis appeared to be decreased in guinea pigs.

B. Animals exposed to intermediate and low concentrations of MEA. Gross and microscopic examination of tissues and organs of dogs and rats did not show any changes that could be attributed to MEA exposure.

Skin Changes

Morphologic skin changes were similar in all species of animals exposed to MEA. Skin of animals exposed to concentrations of from 5 to 15 ppm showed slightly increased epithelial thickness, with increased amount of exfoliated cells. The scrotal and chest skin of dogs exposed to 25 ppm had an uneven surface. Some of these depressions were overlaid by a layer of exfoliated cornified epithelial tissue interspersed with pyknotic nuclei of leucocytes. Cells in the germinal layer contained cytoplasm that appeared vesiculated. The cells overlying these had vacuolated cytoplasm.

In skin from animals exposed to high concentrations (66–102 ppm), areas of vacuolated epithelial cells were more numerous; this condition was most noticeable near areas of ulceration. Plasma passed through the epidermis at these points depositing fibrin. Leucocytes (predominately neutrophils) infiltrated through also, and became a part of the debris. As the epithelium disappeared, infiltration by inflammatory cells became more pronounced, penetrating into dermal tissues among the bundles of muscle fibers. In the more severe lesions, necrosis of dermal tissues extended down to the level of the underlying muscles.

No changes in the hair follicles were noted that would explain the variable thinning of the coat of rats or indicate why large tufts of hair would pull easily from the coats of guinea pigs.

Blood Studies

Results from biochemical studies on the dogs exposed to 102 ppm of MEA are shown in Table IV. There was a plasma protein disturbance as evidenced by a decrease in albumin and increase in globulin. Blood studies showed an increase in white cells with a shift to the left and a relative decrease of lymphocytes. Hemoglobin and hematocrit values decreased.

Results from biochemical and hematological studies on dogs exposed to 5, 12, and 25 ppm MEA showed all values to be within the normal range. At all concentrations of MEA vapor to which rodents were exposed no significant changes in blood values were found.

Odor Detection Test

In addition to the animal experiments human subjects smelled MEA vapor through a Fair-Wells osmoscope. They described the odor as ammoniacal, musty, or foreign, while some were unable to characterize the odor. The concentration detectable by 50 per cent of the subjects was 2.6 ppm, with a 95% confidence interval of 2 to 3.3 ppm. This estimate was based on the response of 12 human subjects. Subjects detected the presence of the vapors by means of a sensation rather than an odor. However, a describable odor was noted at around 25 ppm MEA.

Discussion and Conclusions

The dominant effects of continuous exposure to MEA vapor were skin irritation and lethargy, varying in degree with the intensity of the exposure. The skin irritation may have been a result of the strong alkalinity of monoethanolamine

TABLE IV

Hematology and Blood Chemistry of Dogs Exposed to 102 PPM (0.250 mg/liter) MEA Vapor

		Total protein	Albumin	Globulin	A/G ratio	Hemoglobin	Hematocrit	WBC	Bands	Lymphocytes
		gm %	gm %	gm %		gm %	%	cells/cc	%	%
Control Dog No. 176	Pre-exposure	5.7	3.9	1.8	2.2	14.5	51.7	13,200	4	32
	Intermediate	6.5	4.2	2.3	1.8	13.5	46.2	13,950	4	40
		6.7	4.7	2.0	2.4	13.1	45.1	13,150	1	43
	Final	6.3	4.1	2.2	1.9	14.4	48.5	12,000	2	44
Exposed Dog No. 180	Pre-exposure	6.4	4.1	2.3	1.86	13.9	51.0	17,900	5	15
	Intermediate	5.7	3.6	2.1	1.7	12.3	41.8	29,375	22	11
		4.7	2.3	2.4	0.96	11.5	38.5	42,250	22	8
	Final	4.7	2.2	2.5	0.89	9.6	35.2	33,000	20	7
Exposed Dog No. 179*	Pre-exposure	6.6	4.29	2.3	1.9	14.0	50.5	15,925	1	25
	Intermediate	6.2	3.9	2.3	1.7	—	42.9	—	—	—
		—	—	—	—	10.3	36.3	36,025	18	17
	Final	6.3	3.7	2.6	1.4	9.0	21.9	32,450	22	12
Exposed Dog No. 177	Pre-exposure	6.5	4.4	2.0	2.2	13.9	50.1	12,675	—	23
	Intermediate	5.9	3.6	2.3	1.6	11.5	40.7	24,425	6	13
		6.7	3.7	3.0	1.2	12.8	40.7	23,265	2	13
	Final	6.5	3.4	3.1	1.1	13.1	46.2	18,645	0	19

* Died after 3 weeks exposure.

potentiated by the contact of the skin with surfaces of the chamber. Behavioral changes, similar to those demonstrated by Smyth[4] occurred in dogs exposed to 26 and 102 ppm MEA where they showed progressive stages of excitation followed by depression.

Systemic effects of MEA were seen in dogs exposed to 102 ppm. The changes in the albumin-globulin ratio correlate with the findings of kidney and liver damage. All dogs showed increases in white blood cell counts during the first 23 days of exposure. In two dogs there were increases in bands. These findings were related to the magnitude of their respiratory disturbances and fever. Two dogs showed a continuous decrease in hemoglobin and hematocrit values, indicating that red blood cell formation may have been suppressed. This suppression may have been due to a chronic infection caused by the chronic intoxication.

Only microscopic changes in the skin of rats, guinea pigs and dogs exposed to MEA showed definite trends which could be correlated with exposure concentrations.

Our data are in agreement with Treon's finding[4] that dogs are less sensitive to MEA vapor than are rodents. However, he found that guinea pigs were more susceptible than rats to these vapors, while in our case both species had similar survival times. This difference in rodent sensitivity found between our experiments and Treon's may be due to differences that the metabolic detoxification processes play during intermittent exposure to high concentrations. Another major difference from Treon's work was our finding of signs of intoxication at the lower concentrations. For example, at a concentration of 0.1 mg/liter Treon observed no mortality in rats and guinea pigs when exposed intermittently for 25 days whereas we observed over 75% mortality during continuous exposure to a similar concentration.

These comparisons show that when evaluating the toxicity of a compound, consideration must be given to the duration of exposure when the exposures are at low concentrations. Furthermore, the results from intermittent exposures, at least with MEA, cannot directly be applied to continuous exposure.

We have calculated that a retained dose of between 36 and 48 mg/kg/day caused definite signs of intoxication in our rats who inhaled MEA vapor at a concentration of 12 ppm. These calculations are based on the assumptions that a 250-gram rat inhales between 300–400 liters of air in a 24-hour day and retains all of the compound inhaled. Comparing the calculated retained dose from continuous inhalation of 12 ppm MEA with doses from the feeding experiment of Smyth (320 mg/kg/day) indicates that MEA is at least 10 times more toxic when given by continuous inhalation exposure than by ingestion.

Continuous prolonged exposure of animals to 12 to 15 ppm of MEA caused skin irritation and lethargy; these concentrations should be considered unsafe for man. Since dogs and rats exposed to 5 ppm exhibited physiological effects,

the concentration of MEA in air safe for man under continuous conditions of exposure would be below this level. The extent to which such a safe level is below this level cannot be estimated from these studies.

Summary

Continuous exposure of animals to 12 ppm or higher of MEA caused behavioral effects and skin irritation. Exposure to very high concentrations (66–102 ppm MEA) caused death and produced lung inflammation and liver and kidney damage in dogs, rats, and guinea pigs. Exposure of animals to 5 ppm produced minimal toxic effects. For man, continuously exposed in environments containing 5 ppm (0.012 mg/liter), the greatest danger would seem to lie in the narcotic effect of MEA vapor.

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Ventilation of Coal Mines

THE U.S. BUREAU OF MINES has issued a publication describing ventilating problems in certain mechanized coal mines. Report of Investigation 5560, *Survey of Face Ventilation Practices in Coal Mines,* can be obtained from the Publications Distribution Section, Bureau of Mines, 4800 Forbes Ave., Pittsburgh 13, Pennsylvania.

The use of continuous mining machines is increasing and the attendent hazards are also. The rapid advance of the working faces and the increased liberation of methane from larger expanses of freshly exposed coal creates ventilation problems that were unknown with older methods of mining. Increased ventilation at the face is necessary to control the hazard. The publication describes the problem and the various ventilation practices in the mines.

Toxicity Studies with Octafluorocyclobutane

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THE use of fluorocarbon compounds as refrigerants, aerosol propellants, fire extinguishants, anesthetics, and synthetic resins is well-known. Now, this versatile class of compounds promises to achieve importance in another aspect of our daily lives, namely, our food. Food propellants, contained in pressurized containers, for household use are not new for carbon dioxide, nitrogen and nitrous oxide have served in the garnishing of our desserts. The fluorocarbon, octafluorocyclobutane (OFCB) has recently been developed to supplement the "old guard" propellants over which it has several advantages. However, prior to its introduction a thorough appraisal of OFCB was required to prove safety in the interests of the public health.

Octafluorocyclobutane, boiling at −6°C, is a gas at room temperature, and studies have shown it to be chemically stable and to impart no taste or odor to food. Chemical tests have indicated that it possesses outstanding resistance to hydrolysis. After storage of OFCB with standard, simulated food material for six months at 100°F and 130°F, *in situ* hydrolysis yielded less than one ppm of fluoride per year.

Since OFCB is a gas at room temperature, the conventional animal feeding studies employed for the toxicological evaluation of a food additive had to be modified to accommodate this property. Consequently, in conference with the Food and Drug Administration, it was agreed that repeated inhalation exposures to an atmosphere containing 10 per cent by volume of OFCB should serve to disclose any cumulative toxic effects. Accordingly, a test was designed in which several mammalian species, including dogs, were exposed to 10 per cent OFCB in air, six hours a day, five days a week, for a minimum of ninety exposures. Exposures to evaluate the acute toxicity in rats, the potential for causing anesthesia in mice, and preliminary repeated exposures were also conducted.

Results of a single four-hour exposure of rats at an atmospheric level of 80 per cent OFCB and 20 per cent oxygen reveal the physiological inertness of OFCB in that the rats survived the exposure and were normal in appearance and activity for the twelve-day observation period following exposure. There was no evidence of gross or microscopic pathological changes that were attributable to the OFCB.

The experiment with mice indicated that OFCB does not produce an anesthetic response in this species. The test used to study the anesthetic potential was patterned after the procedure of Robbins[1] in which two mice are exposed for ten minutes to a known concentration of material in an exposure chamber rotating on its central axis at a rate of 14 rpm. The criterion selected for anesthesia is the inability to maintain an upright position for 15 seconds during rotation. In the test with OFCB six mice were able to maintain positional equilibrium, and five showed only slight, transient effects on respiration during a ten-minute exposure to 80% OFCB and 20% oxygen. They continued in normal condition throughout the one-week observation period and were sacrificed without evidence of gross pathological change.

Preliminary repeated experiments showed that nineteen, six-hour exposures to 1.0 per cent OFCB during a four-week period did not result in toxic effects in rats as judged by growth rate, clinical behavior, hematological measurements, organ weights, and pathology. In addition, six-hour exposures of guinea pigs, rats, and dogs to 10 per cent OFCB carried out for four days disclosed neither clinical evidence of toxicity nor tissue changes attributable to OFCB.

Because of the necessity of exposing animals at a high atmospheric concentration and the then limited availability of food grade OFCB, it was necessary to design an exposure chamber which would accommodate an appropriate number of animals but which would also conserve the supply of test material.

The exposure chamber (Figure 1) meeting these requirements and developed for this test was a cubical enclosure with a volume of 1360 liters. The chamber interior accommodated four

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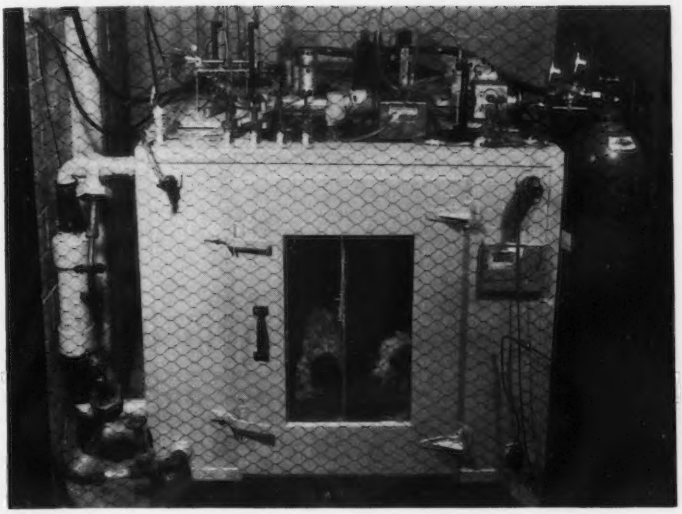

FIGURE 1.

dogs of medium size in the center space and twenty small animal cages on side shelves. Partitions were constructed to separate the animals and to provide attachments for food and water dispensers. On the rear wall of the chamber were mounted a mixing fan and cooling unit to keep OFCB and air concentration uniform within the chamber and to maintain a comfortable ambient temperature, between 24°C and 26°C.

Five sampling valves were mounted on the top of the chamber and four ports were placed on the front surface of the chamber. These permitted access to the chamber for admission, analysis and exhaust of chamber gases and for the measurement of internal pressure.

The animals were placed in the chamber through the door in the front. The door was firmly sealed by means of dog latches to a refrigerator-type gasket. Preliminary leak testing assured that the seal around the door, windows and ports was effective.

A canister device was built onto the side of the chamber and connected to a blower inside the chamber. This system was charged with approximately four pounds of "Baralyme", a mixture of barium hydroxide and calcium hydroxide hydrate, in order to absorb the exhaled carbon dioxide. It was replenished as the level of carbon dioxide in the atmosphere approached one per cent.

The control of the gaseous contents was accomplished by operating the chamber as a "closed-dynamic" system. By this method the gaseous environment was maintained fairly constant by balancing the admission of oxygen and test gas against: (1) the absorption of these gases by the respiration of the animals; (2) the exhalation and removal of carbon dioxide; and (3) the low rate of exhaust of chamber gases.

In operation the sealed chamber containing the animals was primed with oxygen to a concentration of 23 to 24 per cent by volume. This prevented even a slight deficit in oxygen during the admission of OFCB vapor which followed. The loading of OFCB was stopped when the desired concentration was reached, and this point was determined by analysis. During the remainder of the exposure the level of OFCB in the chamber was controlled by admitting a small flow of gas into the chamber. During the admission of any gas to the chamber, an exhaust vent was opened just enough to keep the chamber pressure only slightly positive.

It was necessary to maintain the concentration of other gaseous components within physiologically normal limits. In order to avoid even the slightest anoxia, the concentration of oxygen in the chamber was kept at an average of 18 to 21 per cent by volume by admitting oxygen to the chamber at rates similar to those used to maintain the OFCB level. There was no clinical evidence of anoxia throughout the test. Serious elevation of the CO_2 concentration was prevented by the absorption of CO_2 by the "Baralyme" canister unit. In the present series of exposures, oxygen and carbon dioxide measurements were

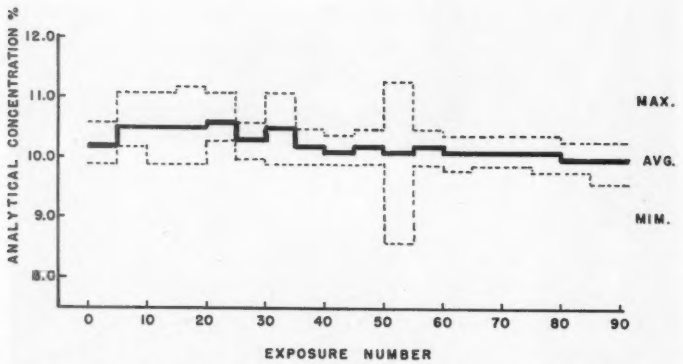

FIGURE 2. Ninety-day inhalation test with 10% OFCB. Average and range of concentrations of OFCB in chamber air. (Each value represents the average of 5 consecutive exposures.)

conducted and recorded continuously for a two-week period, while during other exposures hourly determinations were made. The level of oxygen in the chamber was determined by a Beckman Oxygen Analyzer. Carbon dioxide concentration was determined by a Liston Becker Analyzer. The results of the continuous analyses indicate that the average concentration of CO_2 did not exceed 1.0 per cent and the average oxygen concentration was about 18 per cent.

The distinctive thermal conductivity of OFCB was used as the basis for analysis of the OFCB and air mixtures within the exposure chamber. For this experiment a Gow Mac stainless steel thermal conductivity cell was used. The cell was calibrated by continuous flows of known dilutions of air and OFCB which were verified by Mass Spectrometer analysis. The values obtained from the latter were accurate to within one per cent. For the purposes of the exposures it was decided that limits of ± 0.5 per cent would be satisfactory. Figure 2 illustrates the results of exposure chamber analysis for OFCB. From these data it is evident that the ± 0.5 per cent limit was achieved with only a few exceptions. The overall value was computed as 10.2 per cent OFCB in air.

The animals used in this experiment consisted of 10 male and 10 female rats, 10 female mice, 2 male and 2 female rabbits, and 4 male dogs. In all cases except the dogs, equal numbers of control animals were used. Controls were exposed daily for six hours to a current of air maintained as close to conditions in the test chamber as possible. The small animals were assigned to test and control groups on the basis of body weight and clinical appearance.

The dogs used were mongrels selected for this test on the basis of: (1) adaptability to the chamber environment and to each other; (2) results of measurements of blood pressure, resting heart rate, resting respiration rate, and rectal temperature; (3) results of hematological examination and blood and urine analyses; and (4) body weight and size. When not in the test chamber the dogs were housed in their usual quarters and were given regular exercise in an out-door run.

Results

Before and during the course of the exposures, in addition to the regular measurement of body weight and examinations of individual animals of all species employed, both rats and dogs were subjected to closer study. During the exposure, hematological examinations and urine analyses were conducted monthly on test and control rats. Measurements on the dogs included: blood pressure, resting heart rate, respiration rate, and rectal temperature daily, before and after exposure; hematological examinations and urine analyses before exposure and once every two weeks during the exposures; and determinations every two weeks of the blood concentration of sugar, urea nitrogen, cholesterol, cholinesterase, alkaline phosphatase, and bromsulphalein retention.

At death or sacrifice the major organs of rats and dogs were weighed and the lungs, liver and kidneys of rabbits and mice were weighed. Tissues preserved from all animals for microscopic examination included: brain, lymph nodes, lungs, trachea, heart, liver, spleen, kidneys, adrenals, gonads, stomach, intestine, pancreas, urinary bladder, thyroid, eye, and skin. In addition, the

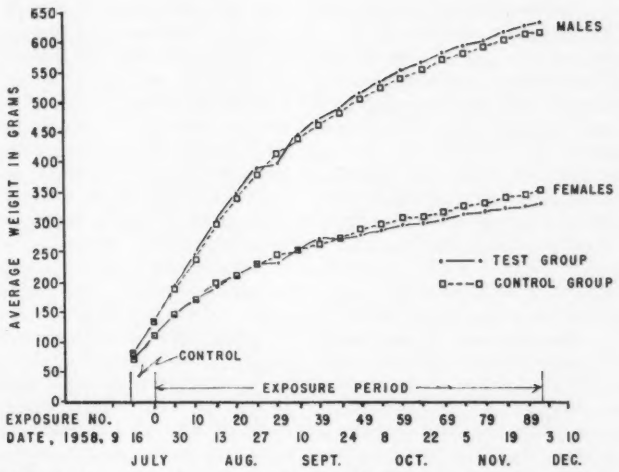

FIGURE 3. Ninety-day inhalation test with 10% OFCB. Growth response of male and female rats.

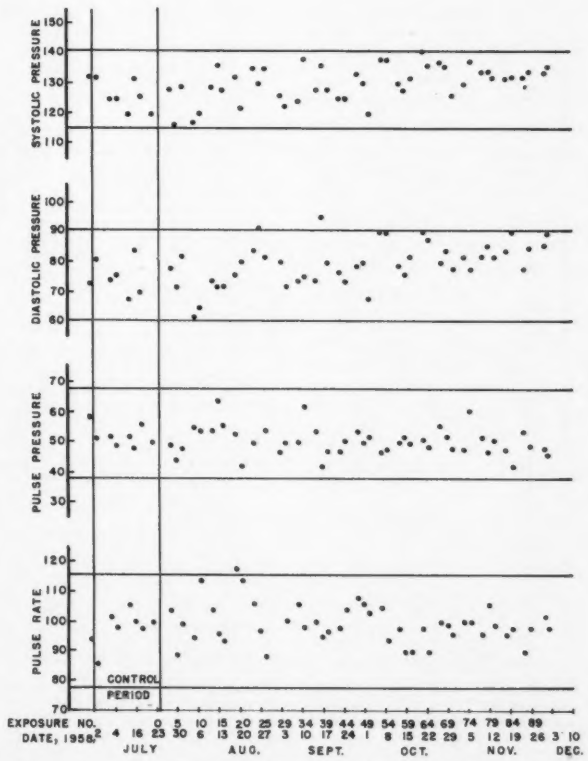

FIGURE 4. Ninety-day inhalation test with 10% OFCB. Blood pressure and resting heart rate of Dog 97.

pituitary of rats and the pituitary, aorta, and striated muscle of the dogs were examined.

The body weight measurements made on the rabbits, mice, and rats indicated that 10 per cent OFCB when inhaled did not affect the ability of these animals to gain weight. Figure 3 illustrates the body weight curves of test and control rats. There was no difference between test and air-exposed controls either in the body weight attained or in the rate of gain exhibited by these two groups. A similar conclusion was reached in regard to the body weights of mice and rabbits. Both of these species showed normal gains in weight throughout the exposure period. The dogs were adult animals and consequently showed no gain in body weight, but they did maintain their weight throughout the exposures.

Clinical observations during exposures disclosed no significant deviations from normal. One mouse died after the third exposure to OFCB because of heavy infestation with parasitic worms. Although this was not expected, since the mice were alleged to have been freed of these worms, it was subsequently learned that they were present in the supplier's colony. Consequently another stock mouse was added to the test group. In the test mice there was also an instance of a prolapsed rectum. In order to prevent cannibalism, this mouse was sacrificed after fifteen exposures without replacement from the stock group. The remaining nine test mice completed the exposure period in good clinical condition. Rats and rabbits exhibited no clinical abnormalities during the exposures.

The appearance of the four dogs as evaluated by activity and condition of coat, gums and eyes remained normal throughout the control and test periods. In order to determine whether the repeated inhalation of 10 per cent OFCB had any effect on the cardiovascular system, measurements of blood pressure, by the indirect method, as well as resting heart rate were made twice daily, once before and once after exposure. The means of four consecutive measurements, systolic, diastolic, pulse pressures, and resting heart rate, were plotted in the form of quality control charts. Pre-exposure values served to establish the normal limits of variation. The lines drawn

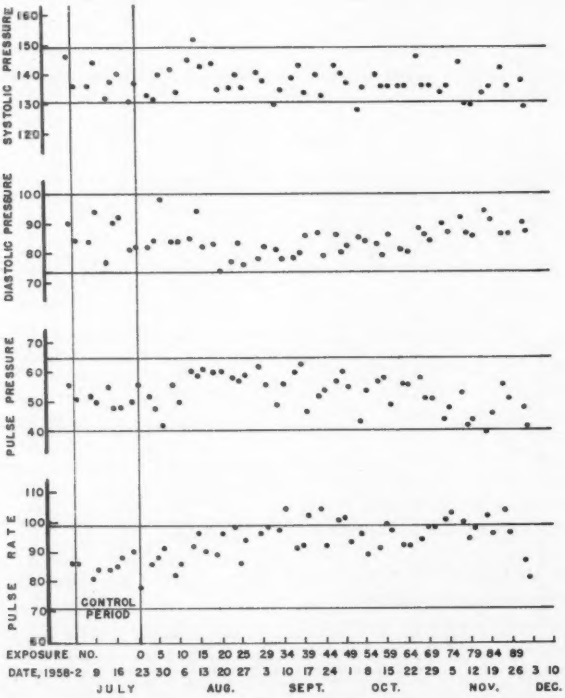

FIGURE 5. Ninety-day inhalation test with 10% OFCB. Blood pressure and resting heart rate of Dog 101.

from this computation represent three times the standard deviation of the means. As illustrated in Figure 4, the measurements of blood pressure, resting heart rate, pulse pressure and rectal temperature, as typified by Dog No. 97, showed only isolated and slight deviations from control limits. An example of one exception to this general observation is shown in Figure 5. In the record of resting heart rate of Dog No. 101, a gradual increase was noted as the test progressed. During this time Dogs No. 101 and No. 99 were frequently fighting in spite of the partitions that separated them from actual contact. On one occasion, because of this behavior, the two dogs were removed from the chamber after approximately one hour, and the remainder of the animals continued on the exposure. In subsequent exposures restraint was necessary to keep the two dogs separate. It is quite likely that this enforcement was responsible for the gradual increase in average resting heart rate in Dog. No. 101, although Dog. No. 99 was apparently not discomfited physiologically. The changes, therefore, were of a temporary nature; and similar to those that have been observed in stock dogs. The values which were recorded at the end of the exposures, other than those assignable to incidental environmental factors, were within normal limits. Therefore, the changes observed are not considered to be attributable to OFCB.

TABLE I

Ninety-day Inhalation Test with 10% OFCB, Summary of Hematology—Rats

	RBC × 10^6/mm		Hb gm/ 100 ml		HT %		Cell size microns		Nucleated RBC/ 100 WBC		WBC × 10^3/mm^3	
	C	T	C	T	C	T	C	T	C	T	C	T
Males												
0	—	—	13.1	13.2	38.0	40.1	7.2	7.5	4.0	5.1	13.82	14.84
30	9.68	9.70	16.9	17.4	49.1	48.0	7.0	6.9	4.4	3.2	15.86	16.95
60	8.93	9.20	17.4	17.7	47.8	49.6	7.1	6.8	0.3	0	14.20	14.64
90	9.26	9.78	17.9	17.7	49.4	49.9	6.6	6.6	0	0	14.14	13.18
Females												
0	—	—	13.5	13.9	39.0	41.8	7.3	7.3	2.4	3.4	13.54	15.62
30	9.31	9.69	16.6	16.9	46.9	47.4	6.7	6.8	1.8	3.7	13.12	12.69
60	8.91	8.82	17.0	17.2	47.5	48.9	6.8	6.7	0.1	0	11.95	10.23
90	9.12	9.06	16.3	16.7	47.4	47.8	6.4	6.6	0.1	0	10.65	9.55

C—control group, T—test group

Table I illustrates the results of the periodic hematological examinations of test and control rats. There were no appreciable differences between control rats and those inhaling 10 per cent OFCB in any of the measurements made before or during exposures. The average white blood cell count of the female rats taken at the end of 90 exposures was slightly lower than that of air-exposed controls, but the difference was not statistically significant.

Similarly in dogs, hematological examinations and measurements of some of the important constituents of circulating blood disclosed no significant departures from pre-exposure values. The red blood cell counts of one dog were occasionally outside pre-treatment limits but these occurrences were not trends toward an abnormal physiological state. There was a tendency for red blood cell counts to be lower and hemoglobin concentration to be higher during exposure than before, but the magnitude of these changes was not inconsistent with a normal physiological condition.

The results of the regular monthly clinical and microscopic examination of the urine of rats showed only slight variations from normal limits. There were no differences between control and

TABLE II

Ninety-day Inhalation Test with 10% OFCB, Summary of Organ Weights of Animals at Sacrifice

(Average Weight in Grams)

	Male rats		Female rats		Female mice		Male rabbits		Female rabbits	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Body wt.	619	636	355	329	33	32	3747	3964	3911	4180
Lungs	1.94	1.83	1.41	1.38	0.23	0.22	11.06	12.10	12.38	12.03
Liver	21.82	22.31	12.02	10.40	1.80	1.82	116	120	125	117
Kidney	3.92	4.08	2.26	2.24	0.44	0.47	15.96	18.88	18.02	18.66
Heart	1.60	1.65	1.03	1.01	—	—	—	—	—	—
Spleen	0.86	0.85	0.59	0.56	—	—	—	—	—	—
Gonads	3.68	3.19	—	—	—	—	—	—	—	—
Brain	2.22	2.22	2.01	1.99	—	—	—	—	—	—

test animals that could not be attributed to random causes. Routine biweekly examination of 24-hour urine specimens from dogs disclosed no differences which would be indicative of an injury sustained through exposure to OFCB.

Results of organ weight measurements are presented in Table II. The weights of the lungs, liver, and kidneys of test rabbits and mice, listed on the right in this table, were similar to those of controls in the adjacent column. Similarly for these organs of the rats there were no significant differences between test and control animals. In addition the weights of heart, spleen, gonads, and brain showed no outstanding differences between test and control rats. Although not illustrated, organ weights of dogs, including in addition to those noted here: adrenals, prostate gland, pancreas, thyroid, and pituitary, disclosed no alteration that would suggest an effect of the exposure to OFCB.

Gross and microscopic examination of tissues from test and control animals after the 90 exposures disclosed no evidence of injury sustained as a result of inhalation of OFCB.

Conclusion

We may conclude in summary that octafluorocyclobutane is a material possessing an extremely low order of acute toxicity. Although Bambach[2] suggested delayed toxic effects as a result of inhalation of 76 per cent OFCB and oxygen stating that "after an indefinite time, animals died from unknown causes", we have observed neither immediate nor delayed signs of toxicity. At 80 per cent OFCB with 20 per cent oxygen there were slight but transient effects on respiration in rats and mice. The studies reported today also show that OFCB does not produce signs of anesthesia or indications of even slight central nervous system depression in the species employed.

The repeated exposures of rats, mice, rabbits, and dogs to an atmosphere containing 10 per cent OFCB for six hours, daily, five days a week for a minimum of 90 exposures resulted in no harmful effects as judged by clinical examination, body weight, blood and urine studies, organ weights, and gross as well as microscopic tissue examination. Since the atmospheric concentration employed in this long-term test is far in excess of that which would ever be encountered in normal use, it is concluded that octafluorocyclobutane is a safe propellant for food products.

References

1. Robbins, B. H.: Preliminary Studies of the Anesthetic Activity of Fluorinated Hydrocarbons. *J. Pharmacol. Exp. Therap. 86:* 197 (1946).
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Forecasting Air Pollution Potential

ON AUGUST 1, 1960 THE U.S. WEATHER BUREAU RESEARCH STATION at the Robert A. Taft Sanitary Engineering Center put into operation a program to provide and disseminate forecasts of prolonged periods of high air pollution potential for all portions of the United States east of the 105th meridian (Rocky Mountains). These forecasts will provide excellent opportunities for special observations by local groups and the study of the influence of meteorologic circumstances. Although the forecasts are experimental, they may serve as an alert to air pollution control officials in affected areas.

To be notified of these forecasts, air pollution control or research units must make arrangements with the nearest Weather Bureau Station. Forecasts in any given area will be issued only when meteorologic predictions indicate periods of high air pollution potential. Further information may be obtained from Air Pollution Engineering Research, Robert A. Taft Sanitary Engineering Center, U.S. Public Health Service, Cincinnati, Ohio.

Studies on the Toxicity of Triethylborane (TEB)

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Introduction

TRIETHYLBORANE (TEB) is an organoboron compound having the empirical formula $(C_2H_5)_3B$ and a molecular weight of 98. It is a clear, colorless liquid boiling at 95°C and has a vapor pressure of approximately 50 mm Hg at room temperature (25°C). The vapors will ignite spontaneously in air at partial pressures of approximately one mm Hg (1300 ppm). Therefore the primary hazard of either liquid TEB or of substantial amounts of vapor is obviously that of fire. The suggested uses of TEB[1] are as fuels and fuel additives for the rocket, missile and aircraft industries; and in nonmilitary applications, TEB has been suggested as a reducing agent, a chemical intermediate, or as a polymerization catalyst.

In view of the well-known toxic effects produced by other boron chemicals, TEB must also be considered as potentially toxic. The vapors of TEB (or its partially oxidized derivatives) can exist in air at concentrations below 1300 ppm without igniting, and the combustion products may pollute the atmosphere with boron-containing smoke. Studies have been performed to determine the magnitude of the potential toxicity of TEB in these terms.

Procedures and Results

Inhalation Studies on Uncombusted TEB Vapors

Triethylborane vapors were prepared by bubbling dry nitrogen gas through an 8.9% (w/w) solution of TEB in mineral oil (Nujol). The volatilization bubbler was maintained at 30°C. By means of this solution of TEB in Nujol, the partial pressure of TEB was lowered so that flammable concentrations were not achieved. The subsequent dilution of the TEB-nitrogen vapor mixture with air was then rapidly accomplished so that spontaneous ignition did not occur. The diluted vapors were passed into a 20-liter gassing chamber which contained the experimental animals. The chamber was operated at the rate of 6 liters/min, which gave a 99% equilibrium time of 15.3 minutes. Different concentrations were prepared by altering the flow of nitrogen through the dispersion bubbler in order to volatilize varying amounts of TEB per unit time. Air, which had been passed over indicating Drierite, made up the difference between the nitrogen flow through the bubbler and the 6 liters/min flow through the chamber. In no case was the volume of added nitrogen more than 10% of the total flow, thereby maintaining an oxygen content of 18.9% or greater in the exposure vapor mixture. Such a decrease in oxygen content was not expected to affect the breathing of rats to a significant extent upon exposure for four hours. Vapor concentrations in the exposure chamber were calculated from the bubbler weight loss, chamber air flow, and time of operation. Nujol has a negligible vapor pressure at room temperature, so calculation of TEB vapor concentrations using the weight loss of the TEB-Nujol solution is permissible.

Six male rats, each weighing between 200–300 gm, were exposed as a group for a single 4-hour period and were observed for seven days following the exposure. Different groups were exposed to various concentrations of the TEB vapor. The observed concentration-mortality data are given in Table I.

It was found that fatally affected animals died either during, or very soon after the exposure. All animals which died within the 7-day observation period were considered to have died as a result of the exposure and have been included in the calculations. The 4-hour LC_{50} was estimated by the method of Litchfield[2] to be 2.80 mg/liter (700 ppm) with a 19/20 confidence interval from 2.67 mg/liter (667 ppm) to 2.94 mg/liter (735 ppm). The slope function of the visually established mortality curve was determined to be 1.062 with a 19/20 confidence interval from 1.025 to 1.102.

The toxicity signs noted for nearly all animals at any of the tested concentrations were increased activity and excitement and "pawing" of the eyes and nose during the exposure. At the

Presented at the Twenty-first Annual Meeting of the American Industrial Hygiene Association, Rochester, New York, April 27, 1960. This research was supported by a grant from Koppers Company, Inc.

TABLE I

Exposure of Rats to Uncombusted TEB Vapor

Concentration of TEB		Mortality	Remarks
mg/l	ppm		
2.36	589	0/6 (0.0%)	No deaths in 7 days.
2.50	624	1/6 (16.7%)	Within 20–24 hours after exposure.
2.70	673	2/6 (33.3%)	One during exposure, one within 18–36 hours.
2.86	714	4/6 (66.7%)	Two deaths during, two within 16 hrs.
3.04	760	6/6 (100%)	Four deaths during, two within 2–18 hours.

higher concentrations (2.50 mg/liter or above) some animals showed frothing at the mouth and/or nose, convulsions and death. Death did not occur in all animals which showed frothing or convulsions. Survivors (those living to the end of the observation period) showed a weight loss following the exposure which seemed to vary with the severity of the response as indicated by toxicity signs and mortality. At the lowest concentration tested (589 ppm), the average weight loss was 5 gm the first day followed by recovery and weight gain. In contrast, at the highest concentration with survivors (714 ppm), the weight loss averaged 10 gm the first day, 4 gm the second day and 2 gm the third day. This weight loss was followed by recovery to the initial weight by the end of the 7-day observation period. Animals which succumbed were autopsied and, on gross examination, showed profuse amounts of fluid, sometimes bloody, in the lungs. Histopathologic examination was not a part of the study.

Exposure to Combustion Products of TEB

For these studies, TEB was volatilized by bubbling dry nitrogen through the pure material. From bubbler weight loss it was determined that saturation was achieved to the extent of about 95% based on a theoretical calculation from TEB vapor pressure data. The "saturated" vapors were directed through an aluminum tube into a burning chamber where the vapors were allowed to undergo spontaneous ignition. The burning chamber was constructed of ¼-inch asbestosite and measured 12 x 12 x 12 inches. This chamber was connected by one-inch brass pipe to a 360-liter dynamic exposure chamber. All exposure air was drawn through both chambers. Concentrations were varied by either volatilizing more TEB or by changing the air flow through the chambers.

Groups of six male rats weighing 200–300 gm each were used at each exposure level. The only toxicity signs noted were "pawing" at the eyes and nares. During the 4-hour exposures, the animals lost as much as 15 gm in average weight, and smaller weight losses were observed in exposures of shorter duration. There was no mortality noted over a 7-day observation period following exposure. The results have been summarized in Table II at the end of this section.

A dense white smoke which appeared homogenous throughout the exposure chamber was produced by the burning TEB. Attempts to collect samples on membrane filter retaining particles above 0.1μ were apparently successful but description of these samples in terms of particle size was not accomplished. The samples were examined under mineral oil and there was not enough contrast between the refractive index of the oil and that of the particular substance to allow description of the samples. Some settling of particles in the combustion and exposure chambers was observed but this did not appear to be appreciable in terms of the amount of TEB combusted.

Spot checks of the exposure chamber atmosphere using an MSA Colorimetric Carbon Monoxide Tester never gave values above 0.002% by volume (20 ppm). This would indicate practically complete combustion of TEB according to the following reaction:

$$2(C_2H_5)_3B + 21\ O_2 \rightarrow B_2O_3 + 12\ CO_2 + 15\ H_2O.$$

Oral Feeding Studies

The 8.9% (w/w) solution of TEB in Nujol (77.9 mg TEB/ml solution, density = 0.872

TABLE II

Exposure of Rats to TEB Combustion Products

TEB combusted[1]		Combustion Products[2]		Exposure time	Temp.[3]	Mortality data	Weight loss[4]
		B_2O_3	CO_2				
mg/l	ppm	mg/l	ppm	min	°F.		gm
0.71	177	0.25	394	240	90	0/6	11
1.20	300	0.43	667	240	95	0/6	15
2.32	580	0.82	1290	60	98	0/6	10
3.15	785	1.12	1750	30	98	0/6	6

[1] Calculated from bubbler weight loss and chamber flow.

[2] Based on calculation from complete combustion reaction (see above.)

[3] Attempts were made to cool the combusted vapors by placing wet rags over the combustion chamber and lead-in pipe. The temperature noted is an average based on 30 min readings in the 4-hour exposures and 10 min readings in the shorter exposures. The greatest observed reading was 102°F.

[4] Average weight loss for six animals during exposure. Weight was recovered within 1–2 days following exposure.

gm/ml) was administered by stomach tube to male rats weighing 200–300 gm each. The assay of this solution was based on boron content as determined by the method of Hill.[3] In the first experiment, there was no mortality or other toxicity sign in 10 rats dosed with 50 mg TEB/kg according to Interstate Commerce Commission requirements. A second experiment produced death within two hours for 5/5 rats dosed with 468 mg/kg. Groups of five rats were then fed intermediate doses in a geometric series (log increment = 0.100) to determine the LD_{50} by this route. The results following a 7-day observation period are summarized in Table III.

It will be noted that as in the acute inhalation tests, animals which died did so very soon after dosing with the TEB. The LD_{50} was estimated by the method of Weil[4] and was found to be 235 mg/kg with a 19/20 confidence interval of 214 mg/kg to 258 mg/kg. The toxicity signs seen at these dosage levels were anorexia and a general lassitude in nearly all animals, and tremors and convulsions in those animals which died. Survivors showed a small (5–10 gm) weight loss for several days following the force feeding of TEB.

Intraperitoneal Injection Studies

A 12.4% (w/w) solution of TEB in Nujol (106 mg TEB/ml solution, density = 0.855 gm/ml), assayed by analysis for B content by the method of Hill,[3] was injected intraperitoneally using a ¾-inch, #22 gauge needle into male rats weighing 200–300 gm each. In the first experiment, five rats were dosed with 2 ml/kg (212 mg/kg) to approximate the oral LD_{50} dose. All convulsed and were dead within one hour. In subsequent experiments, groups of five rats were injected in a geometric series (log increment = 0.301) to estimate the LD_{50} by this route. The dose-response data are shown in Table IV. A 7-day observation period was used in the calculation of results.

TABLE III

Oral Feeding of TEB in Nujol

# Rats	Dose of TEB	Mortality	Remarks
	(mg/kg)		
10	50	0/10	48 hour observation
5	468	5/5	All dead within 2 hours
5	0	0/5	
	(Nujol Control)		
5	159	0/5	
5	200	0/5	
5	252	4/5	All deaths within 2–24 hrs.
5	317	5/5	All deaths within 2–16 hrs.

TABLE IV

Intraperitoneal Injection of TEB in Nujol

# Rats	Dose of TEB	Mortality	Remarks
	(mg/kg)		
5	212	5/5	Within 1 hour
5	0.0	0/5	
	(Nujol Control)		
5	10.6	0/5	
5	21.2	3/5	Within 2 hours-3 days
5	42.4	4/5	Within 1–16 hours
5	84.8	5/5	Within ½–1 hour

Again, as in previous tests, animals succumbed quite rapidly after dosing. The LD_{50}, as estimated by the method of Weil,[4] was found to be 22.7 mgm/kg with a 19/20 confidence interval of 14.7 mgm/kg to 35.2 mgm/kg. The toxic signs were an anesthetized appearance of rapid onset (5–10 minutes), tremors and convulsions. There was no apparent anorexia or significant weight loss in survivors, with the exception that the one animal dosed with 21.2 mgm/kg and which survived for 3 days lost 15 gms before death.

Discussion

Triethylborane (TEB), being a material which spontaneously ignites in air, unquestionably manifests its greatest hazard to life and property in fire. The vapors will ignite above one mm Hg partial pressure (1300 ppm). Since the liquid has a vapor pressure of approximately 50 mm Hg at room temperature, the air layer immediately surrounding the liquid would contain 65,000 ppm of TEB, and would thus be subject to rapid ignition. Small quantities of the liquid or vapor, such as from a minute leak, could be rapidly diluted to below the spontaneous ignition concentration thereby providing a potential exposure to workers or users of the material.

It has been shown in these experiments that inhalation of TEB vapor in concentrations of 700 ppm produces death in 50% of rats exposed for four hours. Such a concentration is classified by Hodge and Sterner[5] as moderately toxic. From these acute experiments, the main toxic effect would seem to be that of respiratory irritation. The convulsions which were seen may be attributed to a direct effect on the central nervous system via pulmonary absorption. Such effect on the central nervous system (CNS) is, incidentally, typical of the effect noted with other boranes. There is the possibility of explaining these convulsions as a secondary effect of anoxia. However, these CNS effects were also seen in the oral and intraperitoneal toxicity

studies where no respiratory distress was evident, thereby giving strength to the argument for a direct effect.

The studies on the combustion products of TEB did not produce any striking toxic effects. The weight loss seen during exposure was undoubtedly due to a loss of body water caused by the elevated chamber temperatures. Measurements were not made of the animal's body temperature. The exposure temperatures placed a limitation on the concentrations we were able to achieve under our experimental set-up. The greater the amount of TEB combusted per unit time, the greater the heat which was produced. Cameron[6] notes that an accidental overnight exposure of rats to 96°F resulted in deaths, which could only be ascribed to the temperature. We did not wish to place a temperature stress on our animals which might have resulted in misleading mortality data.

From the summary table on combustion products, it will be noted that extremely high concentrations were not reached. In fact, at first glance one might assume that they should not have burned at all (based on the 1300 ppm ignition concentration). However, the mechanics of this experiment produced extremely high concentrations of around 65,000 ppm and these ignited before dilution could take place. Strictly speaking, only the combustion products were diluted, not the TEB vapors.

The concentrations of combustion products obtained would not be expected to produce any toxic action unless there was a synergistic effect. The maximum concentrations of CO and CO_2 were 20 ppm and 1750 ppm, respectively, and the threshold limit values for these gases as given by the ACGIH[7] are 100 ppm and 5000 ppm, respectively. Thus there should have been no difficulty from the presence of these substances. Since these experiments have been completed, a report by Wilding, *et al.*[8] states that rats exposed to 0.47 mg/liter boron oxide dust of 2.4μ diameter for 6 hrs/day, 5 days/week for 10 weeks showed only a local irritation of the external nares. Such a concentration produces such a dense cloud that visibility would be limited to 10 to 12 feet. Employees would not likely be expected to work in such an atmosphere for any duration, so likelihood of over-exposure to any of these combustion products is certainly minimal.

TABLE V

Comparison of LD_{50} and LC_{50} of TEB and Decaborane

Route	Compound	mg/kg	mgB/kg
Oral	TEB	235	25.8
	Decaborane	64.3	57
Intraperitoneal	TEB	22.7	2.5
	Decaborane	23.4	20.7
		mg/liter	mgB/liter
Inhalation	TEB	2.80	0.31
	Decaborane	>0.48	>0.42

The oral feeding studies and intraperitoneal studies which were performed using Nujol solutions of TEB are probably only of academic interest since they are not likely to be encountered in industry. The oral LD_{50} of 235 mg TEB/kg would again place the material in a classification of being moderately toxic.[5] The significantly lowered LD_{50} of 22.7 mg/kg and shorter survival time seen when the material was administered intraperitoneally undoubtedly reflects different absorption rates and probably detoxification characteristics of TEB when given by different routes. Since this would not be a natural means of exposure no toxicity rating is established for the intraperitoneal route. However, since such a small amount does produce death by this route, it would seem to indicate that the body is quite susceptible to the material or to its partially oxidized derivatives when it can rapidly reach the site of toxic action. As has been previously mentioned, CNS effects were noted when the material was given by either of these latter routes. Death did not always accompany these convulsions so one might postulate that they were indeed caused by a mechanism comparable to that by which other members of the borane series produce convulsions. Pharmacologic studies would be needed to clear up this point.

If we then accept the postulate that a mechanism similar to that of other boranes is operating to produce the toxic effects for uncombusted TEB, a comparison with similar experiments on decaborane as reported by Svirbely[9, 10] shows TEB to be more toxic than decaborane on a basis of boron content (see Table V). The rationale for making this comparison on the basis of boron content is an extension of the work of Hill, *et al.*[11] which showed a positive correlation between the reducing power of boranes and their toxicity classification based on boron content described by Levinskas.[12] Since TEB is a very strong reducing agent, our findings fit in nicely with their hypothesis.

Conclusions

As has been pointed out, the vapors of uncombusted TEB may be classified as moderately toxic. This classification, together with the fact

that we know nothing of the susceptibility of man or about the cumulative effects or detoxification mechanisms of TEB or the products of partial oxidation, should make it obvious that even low concentrations should not be allowed to exist. It would not be correct to assume that simply because the TEB vapors do not ignite that there is no potential health hazard. The vapor pressure of 50 mm Hg at room temperature is also high enough so that toxic concentrations can be achieved with relative ease.

The recommendations which would seem to be indicated as a result of this battery of studies are first, to keep exposures to uncombusted TEB to a minimum, and second, that if such exposures are inevitable as a result of increased production and usage, repeated exposure studies on several animal species should be undertaken to determine whether chronic effects are produced and at what concentration level with a view toward establishment of safe working conditions or a reasonable threshold limit value.

Summary

A battery of tests has been performed on triethylborane (TEB) to determine some of the characteristics of acute toxic effect. The 4-hour LC_{50} was determined to be 700 ppm. The LD_{50}'s obtained from oral feeding and intraperitoneal injection studies using a Nujol solution of TEB were 235 mg/kg and 22.7 mg/kg, respectively. Respiratory irritation was evident in the inhalation studies, while the other studies indicated a CNS effect. These effects are similar to those seen with other boron compounds and comparison with decaborane shows that TEB is the more toxic on the basis of boron content.

Studies on the toxicity of the combustion products of TEB gave no evidence of any toxic effect over the range tested.

Recommendations have been made to keep exposures to uncombusted TEB vapor to a minimum and to consider repeated exposure studies if increased production and usage of TEB results in the potential exposure of a substantial number of people.

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Air Sampling Instruments

THE AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS through their Committee on Air Sampling Instruments has published a new manual of *Air Sampling Instruments*. Patterned after a similar section in the *Encyclopedia of Instrumentation for Industrial Hygiene*, this new manual brings the information on such instruments up to date. For each instrument uses, operating principle, physical description, performance data, source, operating instructions, calibration, and maintenance are discussed. The manual is divided into the following sections: Impingement sampling devices; Filter media samplers; Impaction devices; Direct reading instruments, Gas and vapor samplers; Colorimetric detectors; Electrostatic and thermal precipitators; and Air sampling pumps. The price is $7.50 per copy and orders should be directed to:

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Acute Inhalation Toxicity of Vinyl Chloride to Laboratory Animals

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Introduction

FIRST prepared in 1833, vinyl chloride has come into increased use in the last decade in the production of polyvinyl chloride resins. The fire and explosion hazard of the monomer and its narcotic properties on acute exposure are well known. As an industrial material, however, it has been regarded as of moderate toxicity. A Threshold Limit Value of 500 ppm was established by the American Conference of Governmental Industrial Hygienists.

No industrial fatalities associated with its use have previously been reported. It was therefore of special interest when two fatalities occurred in a plant handling vinyl chloride. This plant is engaged in the polymerization of the gaseous monomer with the aid of catalysts. One worker died while cleaning out a polymerization vessel and the other while working in a pit. Details of these two fatalities have been reported by one of the authors.[1]

Published information on the acute toxicity of vinyl chloride to experimental animals proved scanty. Patty, Yant and Waite[2] in 1930 reported on the acute response of guinea pigs to vinyl chloride. They found that exposure to 20 to 40 per cent in air killed guinea pigs in a short time; exposure to ten per cent was endured for several hours. Congestion and edema of the lungs with hyperemia of the liver and kidneys were noted.

Peoples and Leake[3] found the minimal anesthetic range of vinyl chloride in mice exposed for ten minutes was 8 to 12 per cent in air; 25 to 30 per cent was fatal in ten minutes. Dogs and rabbits were anesthetized within one minute when exposed to a concentration of about 18 per cent in air. Recovery was very rapid with no apparent untoward effect even after prolonged anesthesia.

Lehmann and Flury[4] noted that vinyl chloride was highly narcotic but had a wide margin between its narcotic and lethal concentrations. Its local irritating effect and its toxicity were described as very slight. Elimination from the body was very rapid. These authors quoted the work of Schaumann[5] with experimental animals. Mice, rats and dogs were able to endure repeated narcosis without any pathological evidence of liver or kidney damage. According to Schaumann, concentrations of seven to ten per cent in air would be narcotic to humans in a short time and 12 per cent would be dangerous.

Vinyl chloride has been studied as a possible anesthetic agent. Oster and associates[6] noted that incoordinated muscular activity of the extremities and cardiac arrythmias of a serious nature were observed in all six dogs used in their experiment. Carr and others[7] found that vinyl chloride produced sensitization of the myocardium in three of seven dogs in which it was administered as an anesthetic agent.

Two cases of vinyl chloride gassing occurred in a factory in Great Britain where polyvinyl chloride resin was being made. These were reported by the Chief Inspector of Factories.[8] In one case a process worker was standing outside a polymerization vessel and washing it with a water stream. After ten minutes of this he suddenly collapsed across the open manhole. Artificial resuscitation was successfully applied. Subsequent symptoms experienced by this worker were tightness of the chest, nausea, abdominal pain and headache. The second case occurred in a maintenance worker who was overcome while repairing a vinyl chloride leak. Both men were hospitalized. Another worker whose hands were accidentally sprayed with vinyl chloride liquid under pressure developed a burn. This latter case was reported by Harris.[9] Such burns may occur with other highly volatile materials when sprayed onto the hands as a liquid under pressure.

Filatova and Gronsberg[10] in 1957 reported on hygienic conditions in a polyvinyl processing plant in the U.S.S.R. The plant was engaged in the polymerization of vinyl chloride by means of

a catalyst. Air sampling was done and the concentration of vinyl chloride was found to vary from about 20 ppm to about 315 ppm. (U.S.S.R. Threshold Limit Value: 1 mg/liter or about 400 ppm). A spastic type blood vessel disorder was described in workers from this plant.

Because of the recent fatalities mentioned above it was decided to undertake acute inhalation studies in laboratory animals with commercially available vinyl chloride. This was done both for comparison with earlier work and to provide information on the acute response and pathological changes in more than one species of laboratory animal.

Experimental Materials and Procedures

Vinyl Chloride: The vinyl chloride was supplied in a metal cylinder under pressure. A commercial grade with the following specifications was used:

Specific gravity at 20°C	0.983–0.985
Water content	None
Boiling range °C	−11 to −9.5
Hydrogen chloride content	None
Acetylene	10 ppm maximum
Acetaldehyde	20 ppm maximum
Iron	Essentially iron free
Purity (per cent)	99.5 maximum
Impurities (per cent)	0.5 maximum

Pure vinyl chloride is a colorless gas at room temperature; its boiling point is −13.9°C. It has a sweetish odor. Its flash point is given at −78°C and its explosive limits in air from 4 to 22 per cent. It has the molecular formula CH_2:CHCl.

Animals: Mice, rats and guinea pigs were used in the study. Test and control animals were taken from the same laboratory stock. All were fed a standard commercial diet and housed in the same way. All were of the same stage of development.

Equipment: The inhalation chamber capacity was 56.6 liters. It was equipped with a viewing window and an inlet tube. Vinyl chloride was released in gaseous form through an adjustable valve on the top of the containing cylinder, then through connecting rubber tubing and a recalibrated Fisher flow-meter. Fresh air was pumped by motor at an adjustable controlled rate through a meter. The streams of air and vinyl chloride were combined at appropriate rates of flow by a glass Y-tube leading through further rubber tubing to the animal chamber inlet, to deliver a continuing stream in the desired proportions. Calculations and adjustments were made to produce the following flow concentrations of vinyl chloride in air for delivery: 10, 20, 30 and 40 per cent. All concentrations are expressed in this report as per cent by volume in air. No determinations of vinyl chloride concentrations were done in the test chamber during the experiments.

Experimental Procedures: Different groups of five mice, five rats and five guinea pigs were placed in the chamber and exposed for thirty minutes to concentrations of 10, 20 and 30 per cent vinyl chloride in air. Similar groups of control animals were maintained but not exposed to vinyl chloride. At the end of thirty minutes exposure the test animals were immediately removed to fresh air. An additional group of five guinea pigs was exposed to a concentration of 40 per cent vinyl chloride in air. In all 65 experimental animals were involved. Observations were recorded on the animals during and after exposure.

The animals which died either during the exposure or after a delay period were autopsied soon after death. Two weeks after the exposure surviving test animals and controls were sacrificed by a blow to the head with the exception of four control rats which were killed by exsanguination.

All animals were examined for gross pathological changes. Tissues were removed from all animals for microscopic examination. The lungs, liver, kidney and heart were removed in all cases. The brain, adrenal, spleen, trachea, lymph nodes, and the eye were removed from representative animals in each group for study. The tissues were preserved in formalin and sections made for staining with hematoxylin-eosin. Special stains were made where indicated.

Observations

Control Animals: No symptoms were exhibited by the 15 control animals and no deaths occurred.

Ten Per Cent Vinyl Chloride: The response of animals exposed to this concentration is recorded below in summary form:

Exposure Time (minutes)	Response
1	Slight irritation in mice and rats.
5	Increased motor activity first in mice, then rats and guinea pigs.
10	Increased motor activity in all species; twitching of extremities in mice.
15	Pronounced tremor, unsteady gait and muscular incoordination in all species.
20	Mice and rats in side position; muscular incoordination, tremors and twitching of extremities in guinea pigs.
25	Mice and rats unconscious, guinea pigs very unsteady but still standing.
30	Mice and rats in deep narcosis; guinea pigs in side position with tremors—one unconscious.

Exposure was stopped and the animals removed to fresh air. All recovered within five minutes.

Twenty Per Cent Vinyl Chloride: The response

of animals exposed to this concentration is recorded below in summary form:

Exposure Time (minutes)	Response
1	Immediate irritation in mice and rats.
2	Muscular incoordination in mice and rats.
5	Mice and rats on their sides with marked tremors and twitching of the extremities; unsteadiness and muscular incoordination in guinea pigs.
10	Mice and rats unconscious with rapid irregular breathing; guinea pigs unconscious but showing marked twitching.
15	All animals in deep narcosis; respirations irregular and rapid.
20	Deep narcosis; breathing slow and shallow.
25	Breathing ceased in one mouse; frothing at mouth and nostrils in mice and rats.
30	All animals in deep narcosis.

Exposure was stopped and the animals removed to fresh air. Mice and rats recovered faster than the guinea pigs and appeared normal within five minutes except for one mouse which was dead. The guinea pigs continued to show muscular incoordination, unsteadiness on their feet until 20 minutes after removal from exposure.

Thirty Per Cent Vinyl Chloride: The response of animals exposed to this concentration is recorded below in summary form:

Exposure Time (minutes)	Response
—	Mice showed irritation immediately and the rats showed irritation quickly thereafter.
1	Muscular incoordination in mice and rats.
2	Mice and rats in side position with marked tremors and twitching of the extremities; muscular incoordination in guinea pigs with developing paralysis of the extremities.
5	All animals unconscious; rapid irregular respirations in mice and rats with frothing about nose and mouth; twitching of extremities still occurred occasionally in guinea pigs.
10	Respirations stopped in mice; breathing slow and shallow in rats; guinea pigs still exhibited occasional twitching movements of the extremities.
15	Breathing stopped in rats; respirations slow and shallow in guinea pigs; twitching of extremities still present in guinea pigs.
30	Mice and rats still; guinea pigs in deep narcosis with slow shallow breathing

Exposure was stopped and the animals removed to fresh air. The mice and rats were dead. The guinea pigs took 25 minutes to return to their normal appearance and activity. One guinea pig from this group died within 24 hours following exposure.

TABLE I

Number of Deaths in Different Groups of Five Mice, Rats and Guinea Pigs Exposed for Thirty Minutes to Varying Concentrations of Vinyl Chloride in Air

Vinyl chloride concentration (per cent by volume in air)	Laboratory animal			Total
	Mice	Rats	Guinea pigs	
10	0/5	0/5	0/5	0/15
20	1/5	0/5	0/5	1/15
30	5/5	5/5	1/5*	11/15
40	—	—	2/5*	2/5
Total	6/15	5/15	3/20	14/50

* A delayed death occurred within 24 hours following exposure.

Forty Per Cent Vinyl Chloride: Only five guinea pigs were exposed to this concentration. Signs of irritation were immediately apparent. Muscular incoordination appeared within seconds. After five minutes all the guinea pigs were unconscious with slow shallow breathing. At the end of the exposure period one guinea pig was dead and the remaining four were in deep narcosis. These survivors took 30 minutes to return to their normal appearance and activity, but one died within the following 24 hours.

The number of deaths occurring in different groups of five laboratory animals exposed for thirty minutes to varying concentrations of vinyl chloride in air is shown in Table I.

Pathological Findings

Gross pathological and histological studies were carried out. The findings are summarized below.

Control Animals: These animals showed no gross or microscopic evidence of damage.

Ten Per Cent Vinyl Chloride: All test animals survived this exposure. They were sacrificed two weeks later. On gross examination there was evidence in mice of slight hyperemia of the lungs. This was less marked in rats. Guinea pigs showed no difference from control animals. On histologic examination the mice showed very slight engorgement of the pulmonary vessels. One mouse showed degenerative changes in the tubular epithelium of the kidney with hydropic swelling. The rats showed slight congestion of the capillaries in the lung. Lungs in the guinea pig were also slightly more hyperemic than those of the control animals.

Twenty Per Cent Vinyl Chloride: One mouse died as a result of 30 minutes exposure to this concentration. Pulmonary congestion was evident on gross examination. Microscopically, there was

engorgement of the blood vessels in the lung with patchy areas of atelectasis and minimal edema. The kidneys showed minimal degenerative changes in the epithelium of the convoluted tubules.

The test animals surviving this exposure were sacrificed in two weeks. On gross examination, congestion of the lungs was present in all species, but it was more marked in the mice and rats than in the guinea pigs. Histologically, there was evidence of pulmonary congestion in exposed animals. Some fatty infiltration was present in the liver of one rat. All other tissues studied appeared normal.

Thirty Per Cent Vinyl Chloride: Gross examination of the animals which died as a result of this exposure revealed congestion of the lungs with hemorrhagic areas. The mice and rats in addition showed congestion of the liver and kidney. The one guinea pig death was delayed. In this animal there was marked congestion of the lungs with hemorrhages and the liver was distended and very friable. The microscopic changes in the animals which died included marked engorgement of the pulmonary blood vessels with edema and hemorrhages in the lungs. The trachea of one rat showed superficial desquamation of the epithelium. Congestion was also evident in the liver and kidney of the mice and rats. The liver of the guinea pig which had the delayed death showed severe fatty degeneration of the liver confirmed with frozen sections stained with Sudan III.

The four surviving guinea pigs were sacrificed two weeks later. Marked pulmonary congestion was present with hemorrhagic areas and edema. In one case there was evidence of secondary bacterial infiltration. The liver in these guinea pigs gave the appearance of fatty infiltration but no fat was demonstrated on frozen section.

Forty Per Cent Vinyl Chloride: One of five guinea pigs exposed to this concentration died during the exposure and another died within 24 hours. The two which died showed marked congestion of the lungs with hemorrhages on gross examination. This was also evident on microscopic examination. The liver of one of these gave the appearance of fatty infiltration, but no fat could be demonstrated on frozen section.

The three surviving guinea pigs were sacrificed two weeks later. Marked congestion of the lungs with hemorrhage was evident on both the gross and microscopic examination. In one guinea pig, the tracheal epithelium was completely absent.

Pathological studies which were made of the brain, heart, spleen, adrenals, lymph nodes and the eyes showed no difference between controls and animals dying as a result of exposure or between controls and the surviving animals sacrificed two weeks after exposure. There was a tendency for the blood to remain unclotted in the animals dying during exposure. This feature was noted in the two human fatalities mentioned earlier but it is not a specific characteristic of vinyl chloride.

Discussion

The response of guinea pigs to inhalation of vinyl chloride was similar to that reported by Patty, Yant and Waite. The guinea pigs in our study, however, tolerated a greater exposure during the experimental period than either the mice or rats. The results in mice agreed closely with those reported by Peoples and Leake. Rats were similar to mice in their response.

The pathological changes in animals which died as a result of exposure were mainly those of vascular engorgement of the lungs with hemorrhages and edema. The severity of these changes varied with the severity of exposure. In the higher concentrations, pulmonary change was marked with severe damage to the tracheal epithelium. Congestion of the liver and kidneys also occurred in test animals. These changes are similar to those reported by Patty and associates.

Evidence of pulmonary congestion was still present in surviving animals sacrificed two weeks after exposure. Patty and associates reported that such changes had disappeared in about eight days

Degenerative changes in the tubular epithelium of the kidney were noted in one mouse dying as a result of exposure and in one mouse sacrificed two weeks after being exposed. Such changes, however, were minimal and not shown by other animals in the group.

One of the guinea pigs with delayed death following exposure showed severe fatty infiltration of the liver. Changes suggestive of fatty infiltration were observed in other exposed animals but not confirmed by frozen sections stained with Sudan III.

Summary

Separate groups of laboratory animals comprising five mice, five rats and five guinea pigs were exposed in an inhalation chamber to concentrations of 10, 20 and 30 per cent vinyl chloride in air for 30 minutes. An additional five guinea pigs were exposed to a 40 per cent concentration for a similar period of time. The response by each of the three species to these concentrations is noted.

Inhalation of these relatively high concentrations produced narcosis and death. Mice were the most susceptible with guinea pigs considerably more resistant. Rats were similar to mice in their

response. Exposure to ten per cent vinyl chloride in air produced deep narcosis in mice and rats but no deaths; 30 per cent concentrations killed mice and rats. Exposure of guinea pigs to 20 per cent produced deep narcosis; but three of five guinea pigs survived exposure to 40 per cent concentration.

Animals dying as a result of exposure were autopsied shortly after death. Survivors and control animals were sacrificed two weeks later. Gross pathological and microscopic studies were done and these findings are described.

The principal pathological changes in animals dying from exposure were congestion of the lungs with pulmonary edema and hemorrhages in some, and congestion of the liver and kidneys. Failure of the blood to clot was also observed. One of the three guinea pigs which died following exposure showed severe fatty infiltration of the liver.

Survivors sacrificed two weeks after exposure showed little difference from the control animals. Pulmonary congestion was still evident but liver and kidney congestion was not.

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Acute and Sub-acute Toxicity Studies of Thallium Compounds

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Introduction

THE element thallium was discovered by Crookes in 1861 by spectroscopic techniques and isolated chemically one year later by A. Lamy. There are no large industrial uses for thallium and its production has been limited. The principal uses of thallium have been related to its rodenticidal properties and it was formerly used as a depilating agent. Thallium salts are also used in the production of certain types of optical glass and in the manufacture of scintillation counters.

An examination of the literature reveals a number of published reports and reviews[1, 2, 3] of thallium toxicity following ingestion of rodenticides containing thallium compounds. A considerable amount of work has been reported on the depilating action of thallium compounds on laboratory animals and man.[4, 5, 6, 7] The distribution and excretion of isotopic thallium (Tl^{204}) has been reported by a number of investigators.[8, 9, 10] Recently, Stavinoha and co-workers[11] have demonstrated that certain compounds containing sulfur act as protective agents against acute thallium poisoning in mice.

The work reported below is concerned with the acute toxicity of thallous acetate (TA) and thallic oxide (TO) following the administration of these compounds to several species of animals by several routes. The ingestion toxicity of these compounds was also investigated for periods up to three months.

Experimental Methods and Materials

The two thallium compounds studied were obtained from the American Smelting and Refining Co., Denver, Colorado (thallic oxide) and Chemical Commerce, Newark, New Jersey (thallous acetate). The purity of these compounds was relatively high (99.5–99.8% Tl).

This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

The thallous acetate was prepared as an aqueous solution at concentrations of 1, 2 or 5 per cent. The thallic oxide was suspended (5%) in distilled water containing 0.8 per cent carboxy methylcellulose.

Young adult, female and male albino rats, originally of the Wistar strain (1923) and bred in the Project Colony were used. The rabbits were young, mature albinos obtained from local breeders. Guinea pigs were young mature animals of mixed sex and obtained from local breeders. The dogs were beagle-type mongrels obtained from local sources.

Single doses of thallous acetate and thallic oxide were administered by intravenous injection to rabbits, by intraperitoneal injection to rabbits, guinea pigs and rats, and orally to rabbits, guinea pigs, rats and dogs. Groups of 2 to 5 rats received single oral or intraperitoneal doses of the compounds. Generally only one rabbit, guinea pig or dog was treated at each dose level. The LD_{50} values, their confidence limits, and the slope function of the regression line, were calculated by the method of Litchfield and Wilcoxon.[12] All animals were held for a 14-day period of observation following injection.

The ingestion studies were conducted using weanling (50–80 gm) albino rats. The basal diet was Purina Fox Chow Meal. The rats were kept in basket-type cages and were permitted to take food and water *ad lib.* The experimental diets were prepared by the admixture of the thallium compounds to the basal diet using an electric mixer. Body weights and clinical data were recorded.

At termination of the ingestion study all of the surviving rats were sacrificed by decapitation. The rats were examined grossly and the heart, liver, kidneys, lungs, spleen, testes and femurs were removed and their weights recorded. Sections of the liver, kidney, brain, lung, spleen, heart, stomach, testes, skin, and adrenals were fixed in Bouins and stained with hematoxylin and eosin for histological study. Portions of the

lung, liver, kidney, spleen, brain and bone were reserved for the spectrochemical analysis of their thallium content.

Thallium was determined by a spectrochemical method employing the direct current carbon arc and a Bausch and Lomb medium quartz spectograph. Regular grade acid purified spectroscopic electrodes were used. The arc was burned for two minutes at a current of 15 amperes. The tissue samples were digested in nitric acid and suitable aliquots taken, the analyses being made without a chemical separation of the thallium. The thallium spectrum line 2767.9 Å is measured. The internal standard is bismuth, one microgram being added to the electrode after the thallium tissue sample is dried. Bi 2898.0 Å is evaluated. The smallest amount of Tl measured in the electrode is 0.2 micrograms and the standard error of an analysis made in triplicate is about ±10 per cent.

Results

Acute Toxicity Studies.

A limited amount of data was obtained following the administration of thallous acetate (TA) by several routes to four different species. The lethal ranges (highest non-lethal dose and lowest dose causing lethality over a 14-day observation period) are given in Table I. The lethal dose for the various species ranged from 7 to 29 mg Tl/kg. No marked difference was apparent with the various routes of administration. The guinea pigs appeared to be somewhat more susceptible to thallous acetate given by intraperitoneal injection. Most deaths occurred between the third and fifth day after treatment.

Thallic oxide, a relatively insoluble compound, also exhibited a high toxicity when administered to the same four species. A surprising finding was the marked toxicity of thallic oxide following oral administration. The oral dose required for lethality was approximately 50 per cent less than that required for lethality by the intravenous and intraperitoneal routes. Here again, the guinea pig was the least tolerant species and this lack of tolerance was noted particularly when TO was given orally. The lowest lethal dose of TO given orally, to the guinea pig, was 5 mg Tl/kg; in other species this dose ranged from 20 to 30 mg Tl/kg. Administered by intraperitoneal injection TO was somewhat less toxic for all species. However, the most susceptible species to TO by this route was the guinea pig. A dosage of 30 mg Tl/kg was lethal to this species whereas the lowest lethal dose for the rat and rabbit was 92 and 60 mg Tl/kg, respectively. A uniform finding was the time of death; most animals died between the third and fifth day following treatment.

The LD_{50} (7-day) for thallous acetate and thallic oxide was determined for the young albino, female rat (120–170 gm) following single oral and intraperitoneal doses of these compounds. These values, their confidence limits ($p = 0.05$) and the slope function of the regression line are given in Table II.

The LD_{50} values for TA were of the same order of magnitude irrespective of the route of administration, and ranged from 23 mg Tl/kg (intraperitoneal) to 32 mg Tl/kg for orally administered TA. Thallium oxide administered orally

TABLE I

Acute Toxicity of Thallium Compounds

Route	Species	Thallous acetate		Thallic oxide	
		No. animals and sex	Lethal range* (mg Tl/kg)	No. animals and sex	Lethal range* (mg Tl/kg)
Intravenous	Rabbit	7 F	12–20	3 F	24–39
Intraperitoneal	Rabbit	9 F	8–13	3 M	30–60
	Guinea Pig	25 M-F	4–7	3 F	10–30
	Rat	41 F	13–20	41 F	62–92
Oral	Rabbit	4 F	12–19	3 M	10–30
	Guinea Pig	4 M	8–12	10 M-F	3–5
	Rat	37 F	18–29	42 F	9–20
	Dog	3 M-F	10–20	3 M	20–30

* Highest non-lethal dose—lowest dose showing any lethality during 14-day observation period.

TABLE II

Acute Toxicity of Thallium Compounds
LD_{50} Determinations on Female Rats

	Thallous acetate (mg Tl/kg)	Thallic oxide (mg Tl/kg)
Single Intraperitoneal Dose		
LD_{50} (7 Days)	23	72
Confidence limits		
Lower	19	28
Upper	29	187
Slope function of regression line	1.23	3.87
Single Oral Dose		
LD_{50} (7 Days)	32	39
Confidence limits		
Lower	25	22
Upper	40	69
Slope function of regression line	1.39	1.76

was somewhat more toxic (LD_{50} = 39 mg Tl/kg) than when given by intraperitoneal injection where the LD_{50} was 72 mg Tl/kg. Considerable variability in the response of the rats given TO by intraperitoneal injection was noted and this variability is reflected in the "flatness" of the response curve. The slope function of this curve was 3.87 a value more than double the values obtained for oral and intraperitoneal TA and oral TO.

Ingestion Toxicity of Thallous Acetate.

Initially, a total of seven groups (5 rats/group) of weanling male rats were placed on a basal diet containing 0 (controls), 0.0002, 0.001, 0.005, 0.01, 0.05 and 0.50 per cent TA. The rats were maintained on these diets for one month. Dietary levels of 0.005 per cent or greater resulted in mortalities ranging from 60 to 100 per cent within 10 days. The rats fed diets containing 0.001 and 0.0002 per cent showed no effects on growth and all survived the one-month feeding period.

On the basis of these preliminary findings, four groups of 10 weanling rats (5 males and 5 females/group) were placed on the basal diet containing 0, 0.0005, 0.0015 and 0.005 per cent TA. The rats were maintained on these diets for 15 weeks. Two additional groups of rats were added to this study several weeks later. These rats were placed on diets containing 0 (controls) and 0.003 per cent TA, respectively. The animals were weighed at weekly intervals and the average growth curves (Figures 1 and 2) were plotted. As can be seen from these figures both male and female rats fed dietary levels of 0.0005 and 0.0015 per cent of TA grew as well as the control rats fed the basal diet. Male rats that ingested the diet containing 0.003 per cent TA exhibited a moderate (average 40–50 gm) growth depression; the female rats grew as well as their controls throughout the 60-day period. The rats fed the 0.005 per cent dietary level of TA showed no marked weight response during the first week on the regimen, but the deaths which occurred within the next week indicated clearly the toxicity of this dietary level.

Increased mortality was observed at dietary levels of 0.003 per cent or greater, in both sexes. The mortality data are given in Table III. There were no survivors among the rats fed the highest (0.005%) dietary level of TA and most of the rats fed this level were dead within the first 14 days of the feeding period. At the 0.003 per cent level, mortalities of 80 and 60 per cent for male and female rats, respectively, were recorded. These deaths occurred between the fourth and eighth week of the study.

Lungs, liver, kidneys, heart, spleen, brain and stomach from the surviving rats were weighed and organ weight-body weight ratios were calculated. The values for all organs were within normal limits except for the kidneys which showed

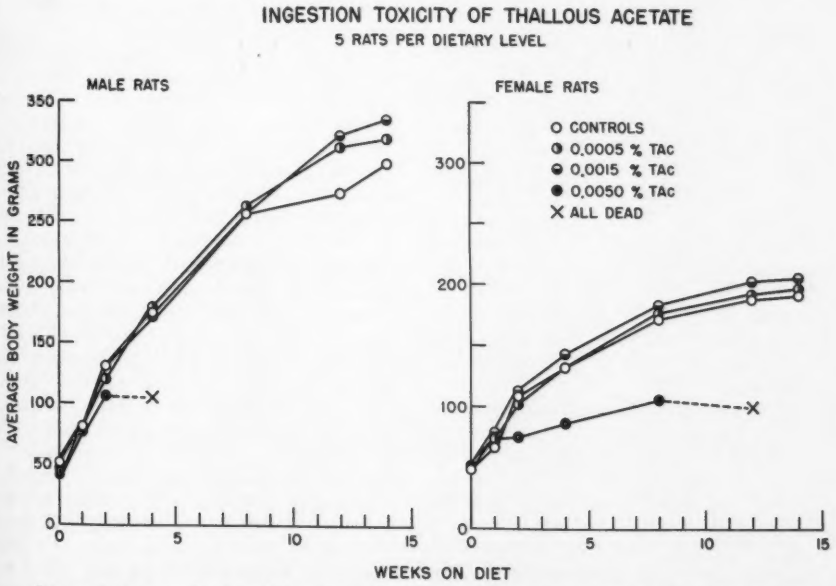

FIGURE 1. Average body weights (gm) of rats fed various dietary levels of thallous acetate.

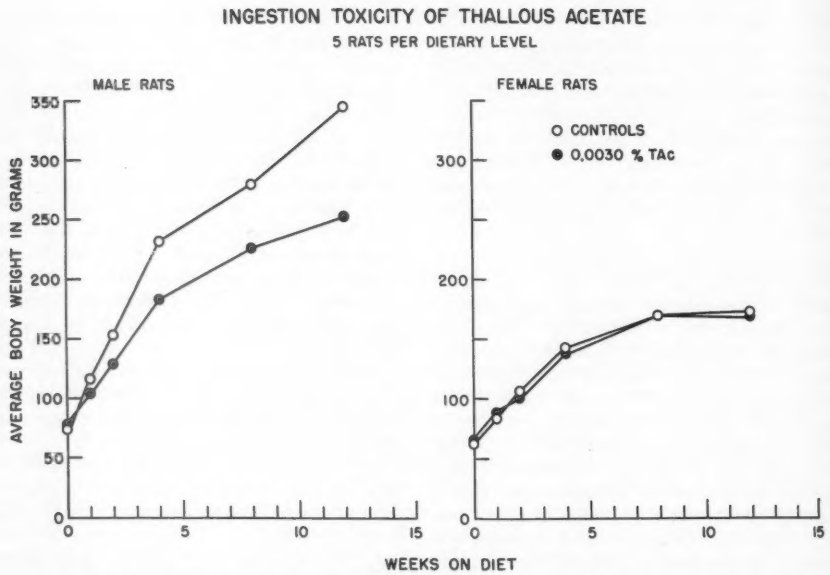

FIGURE 2. Average body weights (gm) of control rats and rats fed a 0.003% dietary level of thallous acetate.

TABLE III

Ingestion Toxicity of Thallous Acetate

Dietary level	Cumulative Mortality									
	Male rats Time on diet (weeks)					Female rats Time on diet (weeks)				
	1	4	8	12	15	1	4	8	12	15
Control	0/5	0/5	1/5	2/5	2/5	0/5	0/5	2/5	2/5	2/5
Control*	0/5	2/5	2/5	—	—	0/5	1/5	1/5	—	—
0.0005%	0/6	2/6	2/6	2/6	2/6	0/4	0/4	0/4	0/4	0/4
0.0015%	0/5	0/5	1/5	2/5	3/5	0/5	0/5	0/5	0/5	1/5
0.0030%*	1/5	2/5	4/5	—	—	1/5	3/5	3/5	—	—
0.0050%	3/5	5/5	—	—	—	0/5	4/5	4/5	5/5	—

* These groups on diet for 63 days.

a slight increase in weight. However, because of the limited number of animals (one to three per group) there is some doubt as to the significance of this difference.

The only major finding on gross autopsy was moderate to marked alopecia in the rats fed 0.0015 and 0.003 per cent TA. This hair loss was noted after two weeks on the diets and at termination the rats were almost free of hair. Microscopic sections of the heart, lung, liver, kidney, spleen, stomach, brain, testes, adrenals, bone marrow, femur and spinal cord showed no histopathological changes that could be attributed to the ingestion of thallium. Skin sections were not prepared from rats fed TA.

Ingestion Toxicity of Thallic Oxide.

Six groups of 10 weanling rats (5 males and 5 females/group) were placed on the basal diet containing 0 (controls), 0.002, 0.0035, 0.005, 0.01, and 0.05 per cent thallic oxide (TO). The rats were maintained on these diets for a 15-week period. The rats were weighed at weekly intervals and average growth curves were plotted (Figure 3). Severe growth depression was observed in both sexes of rats fed dietary levels of 0.005, 0.01 and 0.05 per cent. The male rats fed the 0.0035 per cent TO diet exhibited a marked depression of body weight, so that at termination of the study their average body weight was 180 grams less than the controls. The female rats fed this diet also showed a weight depression, however, this depression was not as marked (average 50 gm) as that observed in the male rats. The male rats fed the lowest (0.002%) dietary level of TO showed a weight depression amounting to an average of 50 grams at termination. This weight depression became apparent only after the fourth week on the diet. The female rats fed the 0.002 per cent diet grew as well as the controls.

Increased mortality was observed in the rats fed dietary levels of 0.005 per cent TO or greater (Table IV). Survival time decreased with the in-

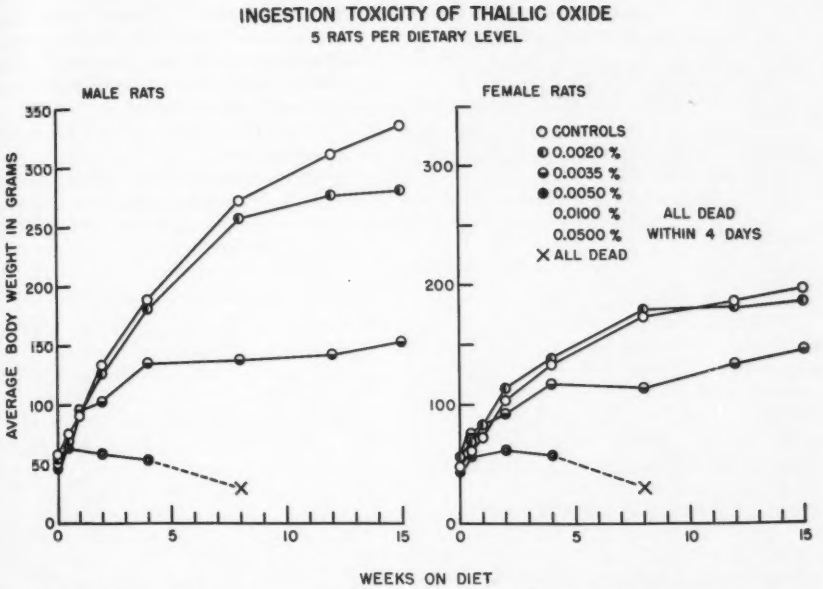

FIGURE 3. Average body weights (gm) of rats fed various dietary levels of thallic oxide.

creased concentration of TO in the diet. Only one male rat survived the 15-week feeding period at the 0.0035 per cent dietary level and most of the deaths occurred after the first month on the diet. The two deaths which were recorded from the female rats fed the 0.0035 per cent dietary level are probably not related to the ingestion of thallium, since the same number of deaths occurred in the female rats fed the lowest (0.002%) dietary level of TO. All male rats fed the lowest dietary level survived the experimental period.

The only gross change observed in the rats fed the 0.002 and 0.0035 per cent TO diets was a marked loss of hair. The hair loss was marked in the male rats after four weeks on the diet and almost complete after six weeks. There appeared to be a greater loss of hair in the male rats when compared with the female rats.

At termination, the surviving rats were sacrificed and the lungs, liver, kidneys, and brain from each rat were weighed and the organ weight-body weight ratios were calculated. The values for the lung, brain, and liver were within normal limits. The kidneys of the rats fed thallic oxide did show an increase in weight (Table V), except for the one rat fed the 0.0035 per cent TO diet. This weight increase was reflected in the kidney weight-body weight ratios. The low kidney weight value for the single male survivor at the highest dietary level is probably related to the small size

TABLE IV
Ingestion Toxicity of Thallic Oxide

Dietary level	Cumulative Mortality									
	Male rats Time on diet (weeks)					Female rats Time on diet (weeks)				
	1	4	8	12	15	1	4	8	12	15
Controls	0/5	0/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5
0.002%	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	1/5	2/5
0.0035%	0/5	0/5	1/5	2/5	4/5	0/5	0/5	1/5	2/5	2/5
0.005%	0/5	5/5	—	—	—	0/5	3/5	5/5	—	—
0.0100%	5/5	—	—	—	—	5/5	—	—	—	—
0.0500%	5/5	—	—	—	—	5/5	—	—	—	—

(terminal weight, 154 gm) of this rat. The differences between the mean kidney weights of the male and female control rats and the rats fed 0.002 per cent TO were found to be statistically significant (t-test) at the 95 per cent probability level ($p = 0.05$), but not significant at the 99 per cent probability level ($p = 0.01$). The "t-test" was also applied in comparing the mean values for the control female kidney weights and the kidney weights of the female rats fed 0.0035 per cent TO. The difference between the means observed was found to be significant at the 0.01 level of probability.

Histopathological examination of sections from

TABLE V

Ingestion Toxicity of Thallic Oxide

Average Kidney Weights and Kidney Weight/Body Weight Ratios

Dietary level of TO	No. rats	Ave. kidney wt. (gm)	Standard deviation	Ave. kidney wt./body wt. mg/gm body wt.
Male rats				
Control	4	2.63	0.18	7.87
0.002%	5	3.24[a]	0.35	11.65
0.0035%	1	2.02	—	13.11
Female rats				
Control	5	1.53	0.04	7.78
0.002%	3	1.85[a]	0.11	9.85
0.0035%	3	2.09[b]	0.12	14.18

[a] Difference from control value is significant (t-test) at $p = 0.05$ but not at $p = 0.01$.

[b] Difference from control value is significant (t-test) at $p = 0.01$.

the lung, liver, kidney, and brain showed no alterations that could be attributed to the ingestion of thallium. However, sections of the skin (H and E stain) did show histological changes.

There was a marked decrease in the number of hair follicles and hair shafts. Considerable atrophy of the remaining follicles was observed and a decrease in the size of the sebaceous glands was noted. The epidermis was keratinized and in some instances, the follicles were cystic. In one or two sections there was some sub-epithelial fibrosis and chronic inflammation. This finding may have resulted from trauma or infection following the loss of the protective function of the hair.

Additional portions of the skin were fixed in (a) neutral ($CaCO_3$) formalin, (b) 10 per cent formalin in absolute alcohol, and (c) in Bouin's fixative and were sent to Dr. Donald Opdyke of the Proctor and Gamble Co., for his examination. Dr. Opdyke's findings[13] are quoted, in part, as follows:

> "There appears to be a total atrophy of the hair follicles, each follicle being replaced by a scar or collagen or fat. There is a general thickening and increase in density of the collagen bundles with a subsequent disappearance of lymph spaces and apparent pressure on blood vessels and follicles. This is not unlike the changes observed in atrophic scleroderma.
>
> "The sebaceous glands appear hyperactive and one is impressed on examining the frozen sections how much excess fat there is on the surface. The sebaceous glands are undergoing involutional changes with frequent dyskeratoses or keratinous degeneration in the gland parenchyma. I would guess that in a short period of time one would find no sebaceous glands at all.
>
> "In the microincinerations there appears to be a surprising depletion of calcium and/or magnesium ash from the cellular areas, particularly from the basal and granular layers of the epidermis and the corresponding regions of the follicles."

Thallium Content of Tissues.

Portions of the kidney, liver, bone (femur), spleen, lung, and brain were reserved from two control rats and two rats fed the 0.003 per cent TA diet. These tissues were analyzed spectrochemically for their thallium content. In addition, kidney, liver, lung, and brain from two rats fed the 0.0035 per cent TO diet were also analyzed. The average values for the thallium content of the various organs are given in Table VI.

The tissues from the control rats were essentially free of thallium. The kidneys from the rats fed the thallium diets contained the greatest (24–31 μg/gm) amount of thallium. The thallium content of the liver and bone was approximately 50 to 60 per cent of that found in the kidney. Smaller amounts (5–9 μg/gm) of thallium were found in the spleen, lung, and brain.

Discussion

A survey of the literature indicates clearly that thallium is one of the more toxic metals. This study confirms this fact.

When thallium is administered in single doses by several routes, doses of the order of 20–50 mg Tl/kg (except for the guinea pig) proved to be lethal. The valence state of thallium does not appear to have any significant effect on the dose required for a lethal effect. When thallic oxide

TABLE VI

Ingestion Toxicity of Thallium Compounds

Average Thallium Content of Rat Organs

Dietary level	No. rats	Micrograms Tl/gm organ (wet wt)					
		Kidney	Liver	Bone	Spleen	Lung	Brain
Control	2	0.0	0.0	0.0	0.0	0.0	0.0
0.003% Thallous acetate (63 days on diet)	2	24	16	19	9	8	5
0.0035% Thallic oxide (106 days on diet)	2	31	13	—	—	8	5

was administered by intraperitoneal injection in the rat somewhat larger doses (90 mg Tl/kg or greater) were required to cause death. It is difficult to say whether this is a species difference or whether this may represent a difference resulting from the route of administration. However, the susceptibility of the rat to thallic oxide when given orally makes this difference rather academic. In this connection, the marked susceptibility of the guinea pig to thallium, given by the intraperitoneal route, as thallous acetate, should be noted. The lowest lethal dose (LLD) for this species was approximately ¼ to ⅓ of the LLD for the rabbit and the rat. In the low dose range (20–50 mg Tl/kg) death generally occurred between the third and seventh day. Early death (24–48 hr) occurred only when considerably larger doses were given. No evidence of alopecia was observed in any of the animals receiving single doses of TA or TO.

Thallium compounds exhibit marked toxicity when ingested. There was no apparent difference between thallium administered as the readily soluble thallous acetate and thallium administered as the relatively insoluble thallic oxide. Dietary levels of 0.005 per cent or greater of either TA or TO were fatal. Growth depression was evident in dietary levels of TA as low as 0.003 per cent. Alopecia was noted in rats fed either compound at dietary levels of 0.0015 per cent or greater. Hair loss increased markedly with the increase in dietary concentration of the thallium compound. This gross change as well as the altered histological state of the skin, was not unexpected since a great deal of attention has been devoted to this particular action of thallium. Nevertheless, the very limited amount of thallium compound required to produce this effect is impressive. Assuming that the rat ingests 20 gms of the 0.0015 per cent diet each day, the total intake for such a rat is approximately 0.3 mg TA per day. This is equivalent to a dose ranging from 1–3 mg/kg/day depending on the body weight of the rat. In spite of the severe alopecia and the histological changes in the skin, no histological alteration was observed in the other organs.

In the rats fed TA and particularly those fed TO, there appeared to be an increase in the kidney weight. The kidney weights in both male and female rats appeared to increase with an increased concentration of TO in the diet. This change in weight was not apparent in the lung, liver or brain of these rats and is difficult to interpret, since no histological changes were observed in the kidneys of these rats.

The tissue analyses although limited in scope, indicate that the kidney is the principal depot for ingested thallium. However, it is clear from these data that thallium does have a wide distribution. This finding is in agreement with the studies reported on the distribution of isotopic thallium (Tl^{204}). For example, Lie and co-workers[9] found that a mean of 5.4 per cent of the body burden (per gram) was present in the kidney at 1, 2 and 7 days after the administration of the isotope. Other tissues such as skin, liver and muscle contained amounts of thallium ranging from 0.033 to 1.0 per cent of the body burden.

Summary

Thallous acetate (TA) and thallic oxide (TO) showed marked toxicity when administered in single doses to rats, rabbits, guinea pigs and dogs by various routes. When given by intraperitoneal injection, single doses ranging from 5 to 20 mg Tl/kg (as TA) were lethal to guinea pigs, rabbits and rats. Administered orally, doses of 20 to 30 mg Tl/kg (as TA or TO) were fatal to rats, rabbits, and dogs. The 7-day LD_{50} for TA given by intraperitoneal injection was 23 mg Tl/kg; the LD_{50} for orally administered TA was 32 mg Tl/kg. The LD_{50} (7 days) for TO administered by intraperitoneal injection was 72 mg Tl/kg; for TO given orally the LD_{50} was 39 mg Tl/kg. When administered by the intravenous route to rabbits, doses of 20 mg Tl/kg (TA) and 39 mg Tl/kg (TO) or greater were lethal.

Feeding studies with weanling rats (5 males and 5 females/group) were conducted over a 90-day period. Rats fed dietary levels as low as 0.003 per cent TA or 0.0035 per cent TO exhibited marked growth depression after 30 days on diet. The major clinical finding at these dietary levels was an almost complete depilation. Histological changes characterized by atrophy of the hair follicles and sebaceous glands were observed in the skin. There were no pathological changes observed in any other organ that could be attributed to the ingestion of thallium. Spectrochemical analysis indicated that the kidney was the principal site of deposition in rats fed diets containing 0.003 per cent TA or 0.0035 per cent TO. The thallium content of the liver was approximately 50 per cent of that found in the kidney. Smaller amounts were found in the spleen, lung, and brain.

Acknowledgment

The authors wish to express their gratitude to Dr. Donald Opdyke, of the Miami Valley Laboratories of the Proctor and Gamble Company for his contribution to the study.

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X-RAYS FROM TV RECEIVERS

THE NATIONAL COMMITTEE on Radiation Protection and Measurement (NCRP) has prepared and released a statement of their position on the maximum permissible dose from television receivers. The position adopted by NCRP is as follows:

"During the past years members of the NCRP have investigated the emission of x-rays from television receivers. From a genetic point of view even sources of minute radiation are of significance if they affect a large number of people. X-rays emitted by home television sets are, therefore, of interest because of the high percentage of the population involved. In order to insure that the television contribution to the population gonad dose will be only a small fraction of that due to natural background radiation, the NCRP recommends that the exposure dose rate at any readily accesible point 5 cm from the surface of any home television received shall not exceed 0.5 mr per hour under normal operating conditions.

"Laboratory and field measurements[1] have shown that with this maximum permissible exposure level the television contribution to the gonad dose at the usual viewing distances will be considerably less than 5 per cent of that due to the average natural background radiation. Most of the present television receivers already meet this requirement with a high factor of safety. In general, therefore, no change in shielding of existing sets will be required. However, the recommended limit will insure that future television receivers, operating at higher voltages, will not contribute significantly to the population gonad dose."

(1) Braestrup, Carl B., and Richard T. Mooney: X-ray Emission from Television Sets. *Science, 130:* 1071–1074 (October 23, 1959).

The Polarographic Determination of Thallium in an Atmospheric Sample

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Introduction

THE determination of thallium by the polarographic technique of Winn, Godfrey and Nelson,[1] which could be applied to atmospheric samples, involves the prior separation of lead by selective extraction and this is time consuming, as is true of a spectrophotometric procedure recently published by Campbell, Milligan and Lindsey.[2] Consequently, the objective of this work was to combine the advantages enumerated by the author[3] relative to the use of calcium chloride as a supporting electrolyte with the effective chelating[4] of any lead present by EDTA to permit the rapid but accurate analysis of thallium and/or lead in an atmospheric sample.

An appreciable increase in sensitivity has resulted from the use of sodium sulfite to reduce the last traces of oxygen to an insignificant level and the polarograms can be obtained without aeration of the solutions by nitrogen which are additional advantages. As illustrated in Figure 1, threshold limit concentrations of either element can be readily analyzed in the presence of each other with an accuracy that has been evaluated by statistical techniques. Larger quantities can be measured even more effectively without dilution or aliquoting which is the major value of the polarographic method.

The extraction of microgram quantities of thallous thallium in the presence of lead and/or iron from 1*N* hydrobromic acid solution by ethyl ether was also studied by means of a factorial experiment.

Equipment

All the calibrated volumetric flasks and pipettes used were freed of lead or heavy metals by washing in a chromic-sulfuric acid mixture followed by rinsing in tap water, 1:1 nitric acid, distilled water and finally redistilled water.

The polarograms were obtained by means of a Sargent Automatic Polarograph Model XXI using a capillary with pre-determined characteristics whose $m^{2/3}t^{1/6}$ value was 2.480 as determined in a non-aerated 0.1*M* potassium chloride solution while the dropping mercury electrode was not shorted to the saturated calomel reference electrode designed by the author.[5] For analytical work the drop time in 2.7*M* $CaCl_2$ (with electrodes shorted) was adjusted to 3.0 seconds and the corresponding mercury level was thereafter kept constant.

Dilutions and solutions were made at 20°C with water redistilled through an all-glass still from CP salts dried to constant weight at 105°C. Stock and standard solutions were discarded at the end of six months usage or when two-thirds of the solution had been used—whichever occurred first. All other chemicals used were reagent grade.

The "no-bump" evaporators were of the Fisher-Moroney type made of cast aluminum, and the stirring apparatus used was of the magnetic type.

Reagents

Acetic acid, 0.2*N*.

Nitric acid, conc: Redistilled in all-glass apparatus.

Calcium chloride, 5.4M: Dissolve 805.0 grams of $CaCl_2 \cdot 2H_2O$ in 1.0 liter weighing the salt in two portions and dissolving each quantity in the minimum amount of water possible. Since the subsequent filtering operation takes 4–6 days, the set-up shown in Figure 2, which is self-explanatory, is recommended.

Bromo cresol purple indicator, 0.04%: Triturate 100 mg of the indicator with 15 ml of 0.01*N* sodium hydroxide and transfer the mixture to a 250 ml volumetric flask using approximately 150 ml of water. If necessary, add more alkali dropwise with mechanical shaking to effect complete solution and adjust pH to 7.0.

Ammonium hydroxide, conc., 1:10, 1:100.

Sodium sulfite, 0.75%: Dissolve salt just prior to use.

Gelatine, 0.25%.

Ethylene diamine tetraacetic acid, sodium salt, 0.1M: Dissolve 9.32 grams in final volume of 250 ml, neutralized to light purple color of bromo cresol purple indicator using ammonia.

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Ethyl ether.

Hydrobromic acid, 48%.

Ferric nitrate, 1.0 mg/ml: Prepared from pure metallic iron.

Thallium solutions (1% in nitric acid).

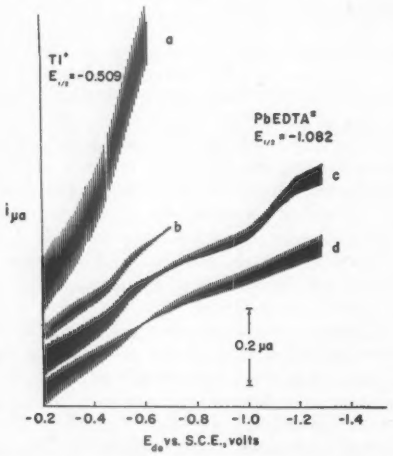

FIGURE 1. Polarograms of thallium (2 μg/ml) in 2.7*M* calcium chloride and 0.005% in gelatine with (a) 0%, and (b) 0.015% sodium sulfite; compared to (c) atmospheric threshold limits of both thallium (2 μg/ml) and lead (4 μg/ml) chelated with EDTA under conditions specified, and (d) residual current for same.

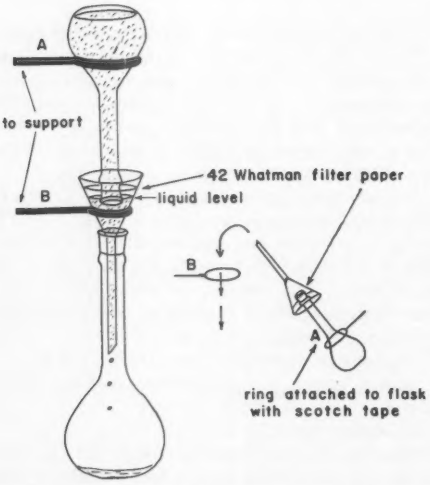

FIGURE 2. Device for unattended filtration of 5.4*M* calcium chloride.

STOCK (≈ 1.0 mg/ml), "M": Dissolve 1.304 grams $TlNO_3$ in distilled water and make to a volume of 1.0 liter.

STANDARDS, M^s (≈ 100 μg/ml) and M^s-1 (≈ 20 μg/ml): Dilute 100.0 ml and 20.0 ml respectively of stock "M" to 1.0 liter.

Lead solutions (1% in nitric acid)

STOCK (≈ 1.0 mg/ml), "N": Dissolve 1.598 grams $Pb(NO_3)_2$ in volume of 1.0 liter.

STANDARDS, N^s (≈ 200 μg/ml), N^s-1 (≈ 100 μg/ml), and N^s-2 (≈ 20 μg/ml): Dilute 200.0, 100.0, and 20.0 ml. respectively of stock "N" to 1.0 liter.

Procedure

Transfer the proper aliquot of the standard metal solution kept at 20°C for 1 hr. or more; or in the case of an actual atmospheric sample, the 5% nitric acid solution (from dissolving the deposits on the tube and electrode of the electrostatic precipitator, or that used in the Greenburg-Smith impinger) to a 250-ml Phillips beaker.

Cover with a Speedy-Vap watch-glass and insert the beaker into a "no-bump" evaporator on Lindberg hot plate. Evaporate solution to dryness, cool, add 5 ml 0.2*N* acetic acid and heat to incipient boiling. Cool, transfer solution to 50 ml volumetric flask using two 10 ml and one 5 ml portions of 5.4*M* calcium chloride to rinse sides of Phillips completely, followed by three small volumes of water.

Add 2 drops of bromo cresol purple, and neutralize to light gray color using approximately two drops of concentrated ammonia followed by 1:10 and 1:100 alkali until amethyst color appears resulting from approximately 2 drops of the latter in excess. Keep flask in constant temperature water bath until whole series is ready for analysis.

Add one ml of 0.75% sodium sulfite, shake well, dilute to approximately 48–49 ml with water and return to bath. A quarter of an hour later, add one ml of 0.25% gelatine, adjust volume accurately, shake well and allow to repose 15 minutes before initiating electrolysis.

If lead is known to be absent, the polarographic cell, D.M.E., and tip of the salt bridge from the S.C.E. are rinsed twice with 10 ml portions of the prepared solution, and then 15 ml are electrolyzed.

In the case of the suspected or known presence of lead, the total prepared solution is transferred to a dry polarographic cell containing a Kel-F plastic covered magnetic bar and while stirring continuously, add 10.0 ml of 0.1*M* EDTA followed by a sufficient quantity of 1:10 ammonia to just restore the purple color, and 2.0 ml of

0.75% sodium sulfite solution. Wipe the S.C.E. tip and capillary with a dry Whatman #42 filter paper (previously washed with nitric acid), and electrolyze the solution. An alternative method is to titrate the lead amperometrically but this is more time consuming, since the pH must be adjusted after each addition of the EDTA.

If iron is also present, 4.0 ml of 48% hydrobromic acid are added to the dried sample in the Phillips flask. After swirling and standing the solution is quantitatively transferred to a 125-ml Squibb funnel using 25 ml of water in portions of 10, 10, and 5 ml. Then, 29 ml of ethyl ether are added, and the mixture shaken for two minutes (allowing pressure to escape from stopcock). The lower layer is discarded and the ether is transferred to the original Phillips beaker using two 5 ml portions of the solvent to rinse the Squibb funnel. Add 5 ml of 0.2*N* acetic acid, cover with Speedy-Vap and evaporate off the ether using no-bump evaporators. The remaining acetic acid solution is then analyzed as indicated above beginning with, "transfer solution to 50-ml volumetric flask. . . . . ."

In all cases, the solutions are electrolyzed under the following conditions, using the Sargent Model XXI polarograph:

Metal	Thallium or lead
Span, V	2.0
Initial, V	−0.2
Recorder sensitivity, μa/mm	0.020 or 0.004
Damping	OFF
Drop time (in 2.7*M* $CaCl_2$), sec	3.0
t°C	20.0

The metal wave height, if any, of a reagent blank prepared from 25 ml of water evaporated to dryness, and treated as outlined is subtracted from the corresponding polarograms obtained with the aliquots of standard solutions, as given in Tables I and II. The corrected data is then used to plot the appropriate standard curves as illustrated in Figures 3 and 4.

TABLE I

The Evaluation of Thallium (alone or in the presence of lead)

Added		Standard curves				Recovery	
Lead, mg/50 ml		0		0		1.0	
0.1*M* EDTA, ml		0		10.0[a]		10.0[a]	
Thallium		Waveheight, mm				Micrograms/ml. found	
ml M^{a}-1	μg/ml	Replicates	Ave.[c]	Replicates[b]	Ave.[c]	Replicates[b]	Ave.[d]
5.0	10.0	9.5	9.63	11.1	11.13	10.6	11.00
		9.0		10.7		11.8	
		10.4		11.6		10.6	
10.0	20.0	19.4	19.57	22.1	22.40	20.1	19.83
		19.9		21.9		21.0	
		19.4		23.3		18.4	
15.0	30.0	30.3	29.77	32.2	33.00	31.2	30.63
		29.1		33.4		29.6	
		29.9		33.4		31.1	
20.0	40.0	40.0	39.97	44.5	44.33	40.6	40.96
		39.3		43.9		39.7	
		40.6		44.6		42.6	
25.0	50.0	50.6	50.47	56.9	56.63	50.6	51.90
		50.4		55.5		50.0	
		50.4		57.5		55.0	

[a] Plus average of 1.2 ml 1:10 ammonia and 2.0 ml sodium sulfite, 0.75%.
[b] Corrected for dilution effect.
[c] Data used for least squares plot.
[d] Joint confidence ellipse calculated from these figures.

TABLE II

Calibration Curve for Determination of Lead as the PbEDTA$^{=}$ Complex

Lead added		Residual Pb††		PbEDTA$^{=}$	
		Waveheight, mm[b]			
ml N[a]	mg/50 ml	Replicates	Ave.	Replicates	Ave.
5.0	1.0	a	0	17.0	17.833
		a		17.4	
		a		19.1	
10.0	2.0	1.9	1.866	35.9	36.300
		1.5		35.8	
		2.2		37.2	
15.0	3.0	3.8	2.700	61.8	61.966
		2.7		60.9	
		2.9		63.2	
20.0	4.0	5.2	4.233	82.2	83.500
		4.6		83.9	
		4.3		84.4	
25.0	5.0	5.2	5.366	107.6	104.000
		5.3		103.0	
		5.6		101.4	

[a] Immeasurable.
[b] Corrected for dilution effect.

Discussion

The main objective of this paper was the development of a technique for the polarographic determination of thallium in the presence of lead using 2.7*M* calcium chloride as supporting electrolyte, and EDTA as a chelating agent. The feasa-

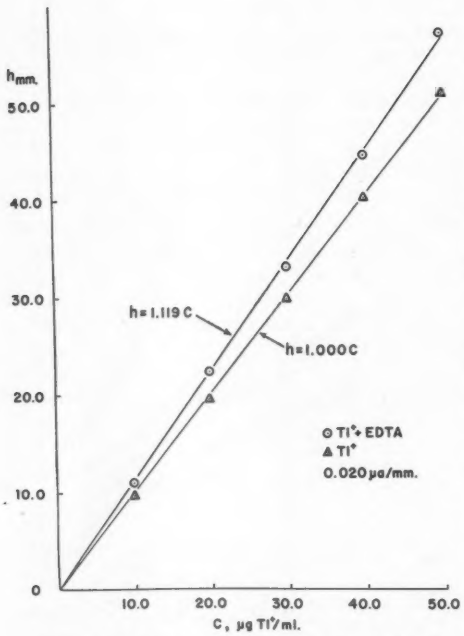

FIGURE 3. Standard curves for determination of thallium in an atmospheric sample, drawn by least squares method.

bility of the latter idea, originally suggested by Pribil and Zabransky but for a different, less concentrated solution, was evaluated by amperometric titration. However, only through compensation for the effect of EDTA, at −0.2V vs S.C.E., on the residual current by adjustment of the latter to a fixed, arbitrary value (after each addition and adjustment of pH to 7.0) was it possible to obtain a differential measurement of the decrease in the lead wave at −0.6V.

As illustrated in Figure 5, a lead concentration of 1.0 mg/50 ml in 2.7*M* calcium chloride required somewhat more than 9.0 ml of 0.1*M* EDTA to effect an essentially complete elimination of the lead interference—even at the high sensitivity employed. If other elements (such as cadmium, nickel, zinc, or manganese) that form complexes with EDTA are present, then the differential amperometric titration should be utilized. Otherwise, time may be saved by simple chelation with 10.0 ml of 0.1*M* EDTA, followed by the adjustment of pH and the addition of 2.0 ml 0.75% sodium sulfite. Consequently, for lead concentrations not exceeding five times the threshold limit, it was found that the original lead wave was reduced to an immeasurable height whereas the lead-EDTA complex (at a considerably more negative half-wave potential) was well defined—especially in the absence of nitrates which is the reason for using acetic acid to dissolve the dried samples.

To verify the observation that up to 20 μg/ml of lead did not interfere in the determination of 10 to 50 μg/ml of thallium, a joint confidence ellipse was calculated according to the technique of Mandel and Linnig.[6] From Figure 6, it may be deduced that a blank correction is not necessary because a line representing b = 0 intersects the ellipse and does in fact lie at its center. Concentrations of lead exceeding 1.0 mg/50 ml would require the application of a correction to the thallium wave as obtained from Figure 3. Secondly, the possibility of a relative-type error was considered negligible since the horizontal line, m = 1 falls well within the ellipse and away from its boundaries.

Consequently, the range of slope values which are compatible with a zero blank as determined from the joint confidence ellipse, extends from 0.988 to 1.071 and since the least squares value for the slope, m = 1.029, falls within these limits, the procedure of not applying a blank correction (in cases up to 1.0 mg lead/50 ml) and dividing

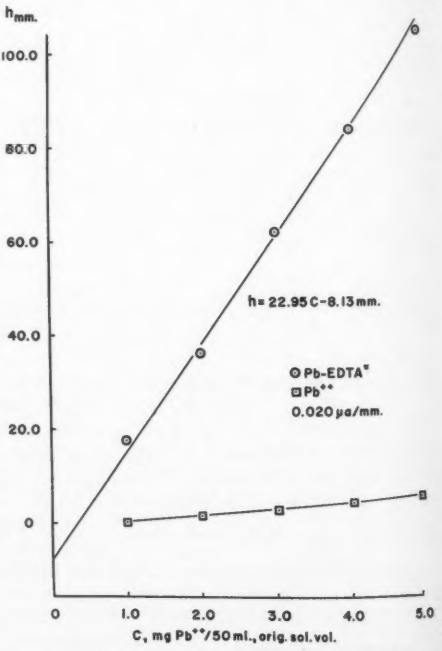

FIGURE 4. Calibration curve for determination of lead as EDTA complex and residual wave.

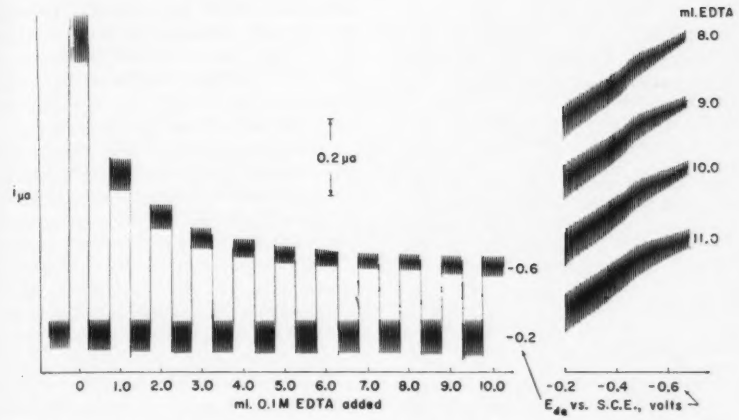

FIGURE 5. Differential amperometric titration of lead (1.0 mg/50 ml) in 2.7*M* $CaCl_2$, containing 0.015% sodium sulfite and 0.005% gelatine at pH 7.0.

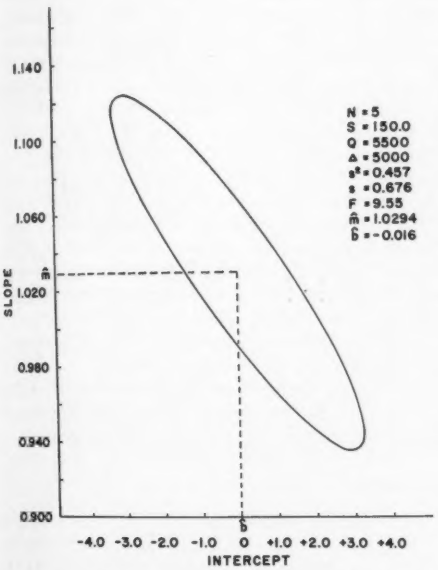

FIGURE 6. Joint confidence ellipse for the determination of thallium (10.0 to 50.0 μg/ml) in the presence of lead (20 μg/ml), using terminology suggested by Mandel and Linnig.[6]

by 1.029 is completely acceptable at the 95% confidence level. Finally, the standard deviation of the slope, which is equal to 0.685 μg Tl/ml indicates that thallium in a range of concentrations varying from 500 to 2500 μg may be determined in the presence of 1000 μg of lead with a standard deviation of 34 μg by following the suggested chelating technique.

Insofar as the final alkalinity of the prepared solution is concerned, Table III demonstrates that any value within a pH range of 6.7 to 8.2 is acceptable based on a 99% confidence interval calculated according to Dean and Dixon's method.[7] However, for standarization purposes pH 7.3 ± 0.3 was utilized, which corresponded to the amethyst color of the indicator under the conditions used.

Relative to evaluating the precision at thallium levels close to the threshold limit, a factorial experiment was set up. All samples were prepared and analyzed in a random manner to obtain the data given in Table IV. It should be noted that the values tabulated are arbitrary because the slopes of the high-range standard curves were used to calculate these results. More realistic data

TABLE III

Effect of pH on Thallium Waveheight

Trial	pH	h_{mm}
1	6.72	19.9
2	6.58	20.3
3	7.33	19.4
4	7.68	19.9
5	7.62	19.4
6	8.18	20.5
7	8.19	20.6

$\bar{x} = 20.00$
$w = 1.20$
$t_w = 0.51$

99% confidence interval[8] = 20.00 ± (1.2)(.51) = 19.4 to 20.6 mm

TABLE IV

Factorial Experiment Demonstrating Effects of Chelating Lead and Extracting Iron on the Determination of Thallium

	Micrograms[a]			
Metal	Added		Added	
Lead[c]	0		200	
Iron[d]	0	5000	0	5000
Thallium	Found[b]			
100	124	93	134	101
100	133	95	119	126
200	214	189	226	140
200	225	174	230	175
400	418	358	424	324
400	405	370	412	330

[a] Recorder sensitivity, 0.004 μa/mm.
[b] Arbitrary values calculated from slope of higher range standard curves (Figure 3).
[c] Sequestered with EDTA, 0.1*M*.
[d] Extracted from 1*N* hydrobromic acid with ethyl ether.

TABLE V

Analysis of Variance in Factorial Experiment

Source	Sum Squares	Degrees Freedom	Mean Square	F
Thallium	293849.329	2	146924.665	1169.55[a]
Chelation	135.375	1	135.375	1.08
Extraction	14455.042	1	14455.042	115.07[a]
Thallium × Chelation	603.005	2	301.503	2.40
Thallium × Extraction	2150.338	2	1075.169	8.56[a]
Chelation × Extraction	495.042	1	495.042	3.94[b]
Thallium × Chelation × Extraction	1210.328	2	605.164	4.82[b]
Residual	1507.500	12	125.625	—
Total	314405.959	23		

[a] Highly significant, $F_{.99}(2,12) = 6.93$
[b] Significant, $F_{.95}(2,12) = 3.88$

would be obtained by using a curve obtained under actual operating conditions (as judged from the slope of the thallium curve calculated from Table IV) but for the formal analysis of variance, this is inconsequential.

Therefore, from Table V it may be concluded that the evaluation of thallium in a concentration range of 300 to 400 μg per aliquot can be effected with a standard deviation of 11.2 μg provided any lead present in concentrations up to 200 μg or twice the thallium threshold limit, is chelated according to the procedure outlined in this paper. The latter conclusion is supported by the insignificant F ratios of 1.08 and 2.40 for the effect of chelation and interaction respectively.

However, the extraction of microgram quantities of thallous thallium by ethyl ether from 1*N* hydrobromic acid according to the technique of Irving and Rossotti[9] for milligram amounts was not complete. This observation is evident from Table IV and confirmed by the highly significant ratios, F = 115.07 for extraction and F = 8.56 for the first order interaction which are given in Table V. Thus, a calibration curve would have to be used under such circumstances.

For the analytical conditions specified, and in concentrations of 1.0 mg/50 ml it was found that stannic(ous) tin did not give a wave, that nickel and zinc, were completely sequestered whereas cadmium and manganese were partially chelated and copper remained unaffected. Since an automatic polarograph was used (with a saturated calomel reference electrode whose resistance was approximately 1000 ohms) no effort was made to apply an uncertain correction for the recorder lag in the following half wave potentials: $Cu^{++} = -0.27_5$; $Pb^{++} = -0.50_9$, $PbEDTA^{-} = -1.08_2$, $Cd^{++} = -0.67_7$, $CdEDTA^{-} = -1.19_0$; $Ni^{++} = -0.96_2$, $Zn^{++} = -1.03_3$; $Mn^{++} = -1.47_0$, but nevertheless these values agree fairly well with those of Khlopin. The EDTA complexes of the latter three elements did not produce measurable waves within the useful range of this supporting electrolyte and the limiting current of the manganese ion had a steep slope but was well defined.

Finally, the usefulness of an adapter designed by the author[10] for the direct tracing of original polarograms on drafting paper with india ink to facilitate reproduction is illustrated in Figures 1 and 6 which were prepared by this technique.

Summary

A technique permitting the rapid polarographic determination of thallous thallium in the presence of lead, at threshold or larger concentrations of either element has been described. The standard deviation of the method, as evaluated by statistical analyses, was found to be 11.2 μg in the threshold limit range of 100 to 400 μg of thallium per aliquot, and 34 μg for concentrations between 500 and 2500 μg of this element. The interference of lead, if present, in quantities up to ten times the threshold limit of thallium, can be completely eliminated by chelation with EDTA and the concentration of the former subsequently determined. In the presence of iron, thallium must be

extracted from 1*N* hydrobromic acid with ethyl ether but due to losses, a calibration curve must be used to calculate the results.

In conclusion, the suggested method permits the rapid analysis of atmospheric samples for thallium without having to isolate this element, and with a precision that falls within the collection efficiency of the usual sampling devices. In addition, the principal interfering ion or lead can be also determined simultaneously on a given sample, which is a decided advantage.

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Scanning Photometer for Spectrographic Plates

THE NATIONAL BUREAU OF STANDARDS has developed an improved scanning photometer to determine wavelengths of spectral lines on a spectrographic plate easily, rapidly, and accurately. Devised by M. L. Kuder of the Bureau's electronic instrumentation laboratory, the instrument optically scans a 0.5 mm wide portion of the spectrographic plate and then presents, on an oscilloscope tube, a curve of spectral line density versus wavelength. A fiducial line on the face of the oscilloscope tube aids in locating the exact center of each spectral line. Wavelengths are determined by measurement of plate displacement from a known calibrating line, such as one of the Hg lines. The phototube sees only a width of 13 microns at any given time, although a total width of 0.5 mm is being scanned, therefore the center of the line can be determined quite accurately. The photometer is adaptable to use of punch cards for recording data for subsequent analysis or printout by a high-speed electronic computer.

Retention and Fate of Iridium-192 in Rats Following Inhalation

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Introduction

THE distribution of iridium-190,192 has been studied by Hamilton and co-workers after intravenous and oral routes of administration.[1, 2] These investigators used iridium in the chloride or oxychloride form. The present report concerns the pharmacodynamics of metallic iridium-192 in rats after inhalation.

Hamilton *et al.* found that during the first two hours after intravenous administration, the material was predominantly in the liver and blood with measurable amounts in other soft tissues. Significant amounts were also found in the urine at short intervals after injection. Although the urinary content decreased markedly by one day after injection, detectable amounts were present for the duration of the experiment (33 days). Liver, spleen, and kidney were found to contain most of the material which was retained in the body at later post-administration time intervals. At seven days after oral administration, small, but appreciable amounts of Ir^{192} were found in kidney, liver, lungs, and spleen. The total overall absorption from the gastrointestinal tract was estimated at about 10 per cent of the administered dose.

In addition to the fundamental interest in the behavior of Ir after administration via the lung, and the significance of this route of entry into the body, metallic iridium was studied with a view to its utility as another relatively "insoluble" radioactive material with which to examine the mechanisms by which particles are transported from the lung.

Procedure

Thirty female rats (Rochester colony; mean weight about 150 gm) were exposed to an Ir^{192} aerosol in groups of six rats. A schematic diagram of the apparatus is shown in Figure 1 and has been described in greater detail elsewhere.[3] The protocol for exposure was essentially that used previously for other materials.[4]

Briefly, the rats were lightly narcotized with 90–110 mg/kg of hexobarbital (Winthrop Laboratories Evipal) and were placed in holders with their snouts fitting snugly into the exposure chamber through slits in rubber covering of the exposure ports (B in Figure 1). Because some contamination of the fur occurred in the initial exposure, in subsequent exposures, the rats were covered with plastic bags which effectively prevented contamination.

An aqueous suspension of iridium, as procured from the U.S.A.E.C. Idaho Operations Office, was placed in an aerosol generator of the aspirator type described by Lauterbach *et al.*,[5] and was attached to a reservoir to maintain a constant level in the generator. Air was passed into the generator through a small hole (#79 drill) at a pressure of 14 psi and a rate of 4.5–6.0 liters per minute.

The mean air concentration was 1.15 microcuries per liter (range—1.09–1.18) or about 1.2410^{-4} μg/liter. Air samples (C in Figure 1) were taken with type AA membrane filters (Millipore) with a sampling rate of 100 cc per minute. The sampling head has been described elsewhere.[3] At E in Figure 1, a sample was taken with a point-to-plane electrostatic precipitator of the type described by Mercer,[6] which deposits particles on an electronmicroscope grid for measurement of particle size. The count median diameter (CMD) was 0.07 μ with a geometric standard deviation of 1.5 (mass median diameter of about 0.12 μ). Air samples and analyses were hampered by the obvious presence of large particle deposits in the aerosol generator, reservoir, and exposure chamber. However, the variations were not considered excessively great under the circumstances of the experiment.

After an exposure of about thirty minutes, the rats were removed and either sacrificed immediately or put into metabolism cages for later sacrifice. Pairs of rats were sacrificed at three hours and at one, three, six, nine, thirteen and fourteen days after exposure in addition to those sacrificed immediately upon termination of the exposure. Major organs and tissues were counted

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in a Texaco Well Counter with an efficiency of about 2.5 per cent. Tissue sections were taken for pathological examination and for autoradiography. A few rats were not used for reasons of obvious contamination and some rats were not sacrificed but were kept for metabolic data beyond 14 days and for further pathological examination.

Results

Deposition

The amount of deposition which occurred was highly variable, as might be expected from the variations in air concentrations from one exposure to another and varying degrees of narcosis and respiratory depression, in addition to normal animal variation and intangible alterations in experimental procedure. However, an estimate of the deposition can be made as follows:

The total material deposited in the rats sacrificed varied from 0.35 microcuries to 5.1 microcuries with a mean value of 2.6 microcuries. If one assumes a 75 ml minute volume for the rat, one arrives at an estimate of the total load presented to the animal by multiplying the total volume inspired by the concentration in the air. Calculating the total dose as a percentage of the total iridium "available" for deposition, an estimated mean deposition value of about 58 per cent was obtained. Although the entire range of deposition was from 10–95 per cent, most values lay between 38 and 60 per cent. Of the iridium deposited, a large fraction appears to have been deposited in the upper respiratory tract (URT). This was judged on the combined content of the trachea and the skinned head of rats sacrificed immediately after exposure. For the entire group of rats, a URT deposition can be calculated under the following assumptions:

1. At short periods after exposure, material in the GI tract represents upper respiratory tract deposition.
2. The first fecal sample at 24 hours after inhalation represents virtually all material deposited in the upper respiratory tract.
3. Absorption of iridium from the GI tract and upper respiratory tract surfaces are not taken into account; that is, there is a tacit assumption that absorbed iridium was primarily from the lower respiratory tract.

The partition of the deposited load was calculated on the above assumptions. It appears that of the material deposited in the rats, greater than 95 per cent of the dose was deposited in the URT. Thus, on the average, less than 5 per cent of the deposited load was in the lower respiratory tract initially.

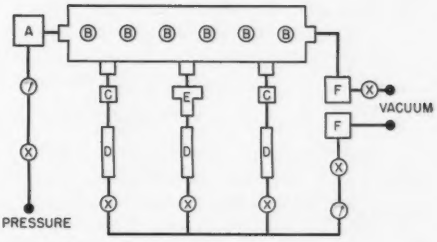

FIGURE 1. Schematic diagram of exposure apparatus.

Retention

Figure 2 is a graph of the disappearance of iridium from the upper respiratory tract. It will be noted that the initial phase of clearance was very rapid, occurring with a half-time of approximately 2–4 hours. The second phase of clearance occurred with a half-time of approximately 24 hours. The 13 day point is barely significant; values at 14 days were not significant.

Lung or lower respiratory tract retention is plotted in Figure 3. Initially, a mean value of only 2.1 per cent of the dose was found in the lung. The material found in the lung immediately after exposure leaves the lung in a manner which is illustrated by a two-phase plot. The first phase

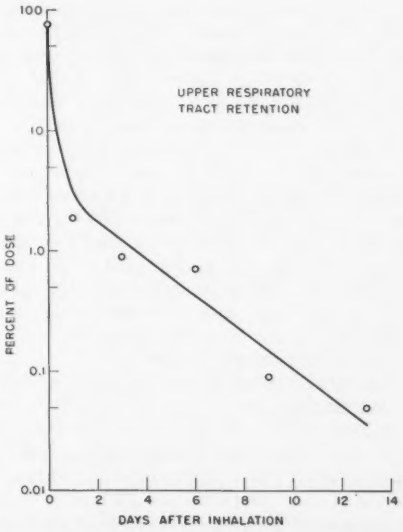

FIGURE 2. Upper respiratory tract clearance.

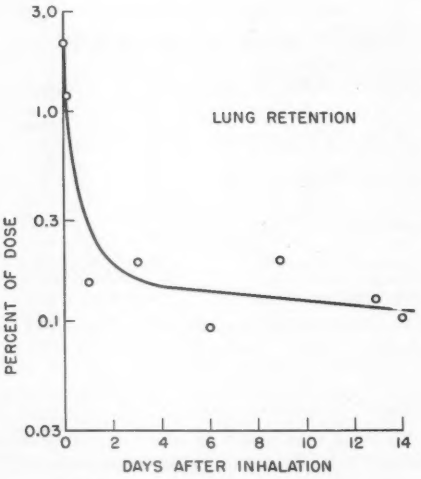

FIGURE 3. Lung clearance.

(0–24 hours) can be represented by a clearance half-time of approximately 6 hours. This probably represents clearance of material deposited in the larger respiratory passages of the lung. Material deposited in the deeper regions of the lung was apparently cleared more slowly as suggested by the second phase of clearance with a half-time of approximately 22–24 days.

Distribution

The redistribution of iridium to other tissues of the body was virtually insignificant except for the gastrointestinal tract load and the material which was found in the residual carcass after tissues had been taken for analysis. There were measurable quantities of iridium in the liver of two rats sacrificed immediately after exposure and one rat sacrificed at three hours post-exposure, amounting to about 0.2–0.6 per cent of the dose. No significant values were found for any other liver samples. Other than two spleen values (0.14% at zero time and 0.02% at 3 days) and two bone values (0.55% and 0.14% of the dose at zero time and 3 hours post-exposure, respectively), no other significant values were found for the tissues analyzed.

One other exception was the skin, in which small, but measurable amounts of iridium were found throughout the duration of 14 days. The skin content is actually reflected in the values for the residual carcass. In Figure 4 the values for iridium content are plotted as per cent of dose in the carcass. If inadvertent contamination was not responsible for the high values at zero time and at 3 hours post-exposure, there is apparently a rapid loss from the carcass initially, with the remainder disappearing at a rate which is represented by an approximate half-time of 7–9 days. Essentially the entire dose of iridium was cleared from the body by the end of the experimental period (14 days). The clearance occurred chiefly via the gastrointestinal tract which contained an average of 46 per cent of the dose at 3 hours post-exposure, about 18 per cent at 24 hours, in addition to that found in the feces at that time, and less than 1 per cent of the dose after three days post-exposure.

Excretion

Over the period of time during which metabolism was followed (28 days), the feces, as might be expected, accounted for most of the iridium excreted. Figure 5 is a plot of the cumulative fecal and urinary excretion. At the termination of the experiment, when nearly all of the material had been excreted, the feces were found to account for more than 96 per cent of the excreted dose while the urinary contribution amounted to ess than 4 per cent. (From animals not yet sacrificed, the total excretion collected has been taken as the "dose," which is virtually correct.)

The rate of urinary excretion when plotted by individual collections from separate animals

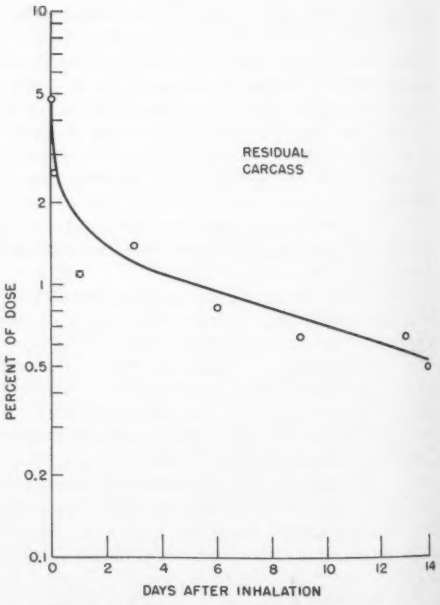

FIGURE 4. Content of residual carcass.

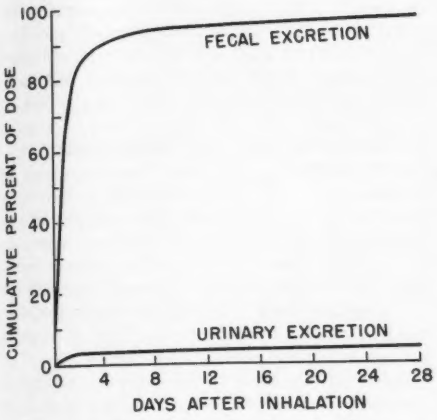

FIGURE 5. Cumulative fecal and urinary excretion.

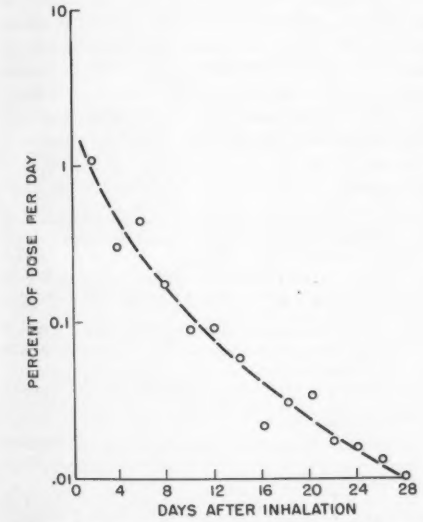

FIGURE 6. Urinary excretion rate. Each point a mean of 48 hour values.

shows a great deal of variability. In Figure 6, 48 hour mean values have been plotted. It is clear that there is not a great deal being excreted by this route, and the major part of that which is excreted in the urine occurs during the first 48 hours.

For the fecal excretion rate, a reasonably good curve can be drawn through the individual points. These data are presented in Figure 7. Note the high rate of fecal excretion shortly after inhalation, the rapid decrease in the rate reaching a level less than 0.2 per cent per day at 14 days after inhalation. A further decrease in rate occurs from 14–28 days post-exposure.

Autoradiography

The quantity of material deposited in the lung was too small to permit construction of a meaningful pattern of movement of particles. In animals sacrificed immediately after exposure, particles were found on the surface of the bronchioles as suggested by the clearance rates. Beyond three hours after exposure, there were small but persistent numbers of particles in the parenchymal regions of the lung. An autoradiographically more useful study would require a larger deposited lung load.

Pathology

The organs examined microscopically were as follows: lungs, major bronchi, trachea, liver, spleen, kidney, gastrointestinal tract, gonads and thyroid. The lungs of two animals (sacrificed at one and six days post-exposure) exhibited foci of chronic bronchitis, bronchiectasis, and pneumonia. The major bronchi from one animal (sacrificed six days post-inhalation) showed chronic ulcerative bronchitis. No lesions were observed in the other organs. The pulmonary lesions differed in no way from the chronic pneumonia commonly observed in this rat colony.

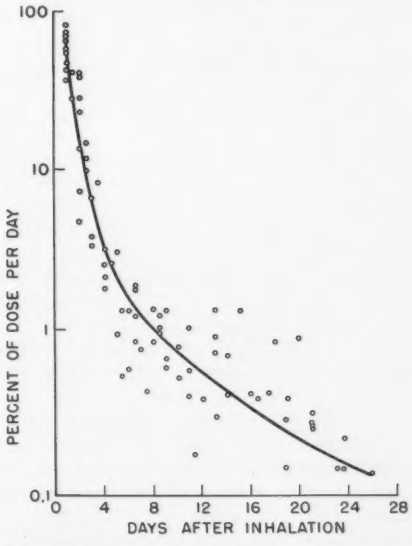

FIGURE 7. Fecal excretion rate. Each point an individual collection and analysis.

No significant pathological effects have been observed through a six month period after exposure.

Discussion

It is quite clear that the major fraction of the deposited material is deposited in the upper respiratory tract. Such a large URT deposition is not readily understood with a particle size distribution such as that found in this experiment (CMD = 0.07 μ). Recently, in this laboratory, using a sodium chloride vector for Po-210, URT deposition values approaching 60 per cent have been obtained with a small particle population (0.046 μ CMD).[4] Similar values have been obtained for particles of slightly larger size in a similar system (0.098 μ CMD).[7] Also, in this laboratory, Boecker[3] has found, with a smaller total deposition, about 50 per cent of the deposited material in the URT with a larger, relatively insoluble PuO_2 particle population (CMD = 0.19 μ).

Assuming the results of the particle size analysis to be correct, one would have to conclude that a great many very small particles were deposited in the upper respiratory tract. Based solely on particle size, one might expect a greater fraction to be deposited parenchymally. Although the deposition of very small particles in the upper respiratory tract is not without precedent, there is no readily apparent explanation of the present results. A more careful examination of the physical chemical properties of the iridium suspension might permit a more meaningful relation of the particle population to deposition.

There is no doubt that the URT load is rapidly removed via the GI tract. Some material apparently leaves the lung rapidly also. As suggested above, and as partially confirmed by autoradiography, this is probably material deposited in bronchi and larger bronchioles. Material deposited in parenchymal regions is cleared at a slower rate.

The excretion pattern is quite clear. The major route of excretion is by way of the feces, amounting to a total of about 96 per cent of the dose which is, coincidentally, similar to the 94–96 per cent of the dose deposited in the upper respiratory tract. Only a small amount appeared in the urine over the experimental period, and this corresponded to what appeared to be distributed to the tissues shortly after inhalation (24 hours). The quantities of material redistributed to organs and excreted in the urine in this study are much less than those found by Hamilton after intravenous injection and oral administration of iridium trichloride.[1, 2] Certainly, the absorption from the GI tract did not approach the 10 per cent found by Hamilton[2] seven days after administration. The differences among these studies is probably a reflection of the greater insolubility of the metallic form of iridium used in this present study over the chloride or oxychloride form of iridium which was used by Hamilton *et al.*

In terms of either the chemical or radiation dose, it would appear that the GI tract was, by far, the most vulnerable tissue after inhalation, followed by the respiratory tract. From this study, other tissues appeared to be subject to insignificant doses by comparison. It should, of course, be pointed out that such conclusions apply only to the rat under these circumstances and may not be directly applicable to a human exposure. It is also clear that iridium particles, if properly sized, would be useful in the study of lung clearance mechanisms.

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Treatment of Chronic Mercurialism with N-Acetyl-Penicillamine

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THE usefulness of chelating and complexing agents in the treatment of heavy metal intoxications has received considerable attention since the wartime development of dimercaptopropanol (BAL) as an antidote for poisoning by arsenic compounds. BAL has been found promising in the treatment of acute mercury, as well a arsenic, intoxication. Soon after World War II, studies in Switzerland[1] and the United States revealed that EDTA (ethylene diamine tetraacetic acid), given intravenously, enhances greatly the excretion of lead and plutonium in a complex form. These findings have been amply verified, but both these agents have certain drawbacks. EDTA is not effective in mercury poisoning; BAL is beneficial only by parenteral administration, is poorly tolerated and has been found effective only in acute mercury poisoning.

Recently it became known that penicillamine given by mouth increases the elimination of metals such as copper. Walshe[2] first suggested the substitution of penicillamine for BAL in the treatment of Wilson's disease following experiments which indicated that penicillamine (β,β-dimethyl cysteine) in oral doses of 300 mg three times daily caused a twenty-fold increase in the urinary copper excretion of normal individuals. He found also a marked increase in copper excretion in six patients with Wilson's disease who were given daily doses of from 0.5 to 1.5 grams. In his short term experiments he did not observe any toxic reaction with this drug and suggests, "On theoretical grounds dimethyl cysteine may well be of use in the treatment of heavy metal poisoning with gold or mercury." Aposhian and associates[3] have suggested N-acetyl-penicillamine as a drug which might be effective against mercury poisoning, based on their studies of its protective action against the acute effects of mercuric chloride in rats.

The following data were obtained with a preparation of N-acetyl-penicillamine powder, given by mouth in ½ gram doses up to 3 grams daily.

Preliminary toxicity tests were carried out on two volunteers who took two capsules (1 gram) daily for seven days without side effects. The urine of one control showed a slightly elevated lead and mercury content with normal concentrations of calcium, zinc and copper.

Cases of Chronic Mercurialism

1. A neighborhood complaint brought to our attention a laboratory used for the purification of mercury. The proprietor and sole occupant (G.D.) was a 75-year-old retired teacher of chemistry who had pursued the purification of mercury for many years. Air tests with the G. E. Mercury Vapor Detector in the laboratory revealed mercury vapor concentrations averaging 1 mg per cubic meter of air. This value is ten times the generally accepted maximum allowable concentration.

The patient presented some of the characteristic signs of chronic mercurialism, all referring to the central nervous system. His gait was unsteady, his hands showed a coarse tremor, and he exhibited mental changes, such as sudden bursts of ill temper. His writing, shown in Figure 1, illustrates the degree to which his tremor had developed. A thorough medical check up at a hospital failed to reveal evidence of gastrointestinal or renal abnormalities. Although in poor general health, he was at first able to carry out the activity necessary to his job, which he insisted upon doing despite all warnings.

A urine sample from this man analyzed according to the micrometric method of Stock and Lux[4] was found to contain 5 mg of mercury per liter of urine, adjusted for the specific gravity. An earlier sample showed 15 mg per liter, but the container was believed contaminated. During this period and prior to it G.D. had worked fairly steadily in his laboratory. Subsequently his activity in the laboratory was reduced. The urinary excretion pattern for about a month prior to receiving acetyl-penicillamine indicated

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FIGURE 1. Sample of a drawing and handwriting of patient, G. D., on June 26, 1959.

an over-all downward trend, although there was a considerable amount of irregularity. His urinary mercury excretion varied during this time from 0.5 to 2.0 mg per day, with an average of 1.3.

Two courses of oral administration of N-acetyl-penicillamine were given. In the first, 1 gm of the drug was taken daily for seven days; in the second, 2 gm per day were taken for three days. There were no signs of side effects nor of changes in his nervous disorder. The mercury excretion was somewhat increased, as shown in Table I.

The patient suffered a mild cerebrovascular attack after termination of the second course of treatment, resulting in weakness in the use of his right arm and leg. For a short period he seemed to improve slightly, but his physical and mental condition continued to deteriorate, and he expired about three months later. An autopsy was performed, and several of the organs were analyzed for mercury content. The kidney, liver, spleen and blood contained respectively 15, 1.6, 0.9, and 0.03 mg of mercury per 100 grams of specimen. The mercury content of various sections of the brain ranged from 0.2 mg to 3.5 mg per 100 grams of tissue, the highest value being in the cerebellum.

2. A 37-year-old mechanic employed at a power plant for three years was exposed to mercury in connection with cleaning and maintaining mercury generators. There was no evidence of ill health prior to December, 1959, when a break in a pipe occurred, exposing him and several other workers for a few hours to high atmospheric concentrations of mercury. Urine samples taken shortly after the exposure on January 15, 1960, and analyzed by another laboratory, showed 1.7 mg of mercury per liter and on February 29, 1960, 0.8 mg per liter.

About January, 1960, the worker began to complain of metallic taste, diarrhea, tendency to nervous tension, and insomnia. Observation at the New England Medical Center in April revealed a slightly tense man with a fine tremor of his hands who seemed otherwise in good health. Laboratory tests showed an elevated blood iodine, suggestive of a mild hyperthyroidism. Renal function tests were normal.

The average urinary mercury excretion per day over a three-day period just before treatment with acetyl-penicillamine was 0.61 mg. During treatment with 3 grams daily of acetyl penicillamine for three days, the average daily excretion of mercury was 0.80 mg (Table II).

Mercury Workers Without Clinical Evidence of Mercurialism

Two fluorescent-lamp workers with a moderate mercury exposure but no evidence of mercurialism

TABLE I

Excretion of G. D. during Treatment with N-Acetyl-Penicillamine

Dates	Dose	Urinary mercury excretion—average		
		mg/day	mg/liter*	mg/gm creatinine
6/13–6/19	None	1.27	2.90	1.70
7/11–7/18	None	1.20	2.54	1.90
7/20–7/26	1 gm./day	2.12	3.38	2.15
7/28–7/30	None	0.87	2.12	1.16
7/31–8/2	2 gm./day	1.38	2.80	1.41
8/3–8/6	None	1.30	2.00	1.14

* Adjusted to a mean specific gravity of 1.024.

TABLE II

Excretion of R. B. during Treatment with N-Acetyl-Penicillamine

Dates	Dose	Urinary mercury excretion—average		
		mg/day	mg/liter*	mg/gm creatinine
4/23–4/25	None	0.61	0.89	0.35
4/26	3 gm.	0.51	0.61	0.28
4/27	3 gm.	0.93	1.05	0.52
4/28	3 gm.	0.96	0.85	0.43

* Adjusted to a mean specific gravity of 1.024.

TABLE III

Excretion of T. J. during Treatment with N-Acetyl-Penicilliamine

Dates	Dose	Urinary mercury excretion—average		
		mg/day	mg/liter*	mg/gm creatinine
12/18	None	0.18	0.19	0.09
12/19	3 gm.	0.44	0.39	0.17
12/20	3 gm.	0.38	0.42	0.17
12/21	None	0.27	0.25	0.11

* Adjusted to a mean specific gravity of 1.024.

TABLE IV

Excretion of N. S. during Treatment with N-Acetyl-Penicillamine

Dates	Dose	Urinary mercury excretion		
		mg/day	mg/liter*	mg/gm creatinine
12/18	None	0.17	0.22	0.10
12/19	3 gm.	0.38	0.28	0.19
12/20	3 gm.	0.28	0.22	0.13

* Adjusted to a mean specific gravity of 1.024.

were each given three grams of N-acetyl-penicillamine daily for three days. The mercury excretion before, during and after treatment is shown in Tables III and IV. As much as a two-fold increase was noted.

Lead Poisoning

Acetyl-penicillamine was also given to a bridge repairman with lead poisoning. He first received 1½ grams per day of N-acetyl-penicillamine for three days and then 3 grams for one day. The urinalysis results are found in Table V. While the enhanced lead excretion resulting from the 1½ gram dose was not very great, it was found that the three gram dose doubled the pretreatment urinary excretion of lead.

Discussion

The increase in mercury excretion following oral administration of acetylpenicillamine has been summarized in Table VI. While the increase is significant, it is not as great as has been observed with other complexing agents and other metals.

Thus, Foreman[5] found a 10 to 100 fold increase in plutonium excretion during administration of EDTA. Numerous investigators have found a 10 to 40 fold increase in lead excretion caused by the same drug.[6] Boulding and Baker[7] found a four-fold increase in lead excretion following administration of penicillamine. Our own experience with acetyl-penicillamine in one case of lead poisoning showed a two-fold increase.

In cases of chronic poisoning by heavy metals (with the possible exception of lead), the clinical effects of complexing agents have not been striking. When a long-lived radioactive isotope has been absorbed, any safe steps to decrease the body content would probably, in the long run, be beneficial. With chemical poisons, the situation is not quite so clear. Removal of the element from sensitive tissues is presumably desirable; whether or not removal from storage areas, such as the bone, has any beneficial clinical effects is open to question. It seems that the therapeutic use of complexing agents is of greatest value in acute chemical poisoning or flare-ups in the course of chronic intoxication, somewhat less valuable in absorption of radioactive substances, and still less in the case of chronic chemical poisons.

TABLE V

Excretion of P. J. during Treatment with N-Acetyl-Penicillamine

Dates	Dose	Urinary lead excretion—average		
		mg/day	mg/liter*	mg/gm creatinine
11/4–11/5	None	0.28	0.32	0.18
11/6–11/8	1½ gm./day	0.33	0.43	0.25
11/9–11/10	None	0.33	0.37	0.22
11/18	None	0.29	0.38	0.21
11/19	3 gm.	0.53	0.74	0.38
11/20	None	0.20	0.30	0.13
12/3	None	—	0.43	0.17

* Adjusted to a mean specific gravity of 1.024.

TABLE VI

Average Per Cent Increase in Mercury Excretion During Treatment

Subject	Dose	mg/day	mg/liter*	mg/gm creatinine
G. D.	1 gm/day	90	52	53
G. D.	2 gm/day	23	26	1
T. J.	3 gm/day	80	84	70
N. S.	3 gm/day	94	13	60
R. B.	3 gm/day	31	−8	18

* Adjusted to a mean specific gravity of 1.024.

In comparing different complexing agents, one is tempted to assume that the one which removes the largest quantities of the harmful agent is the most effective in therapy. While this may be true in the majority of cases, it does not necessarily follow, especially with acute poisoning. Failure of N-acetyl-penicillamine to increase dramatically the concentration of mercury in the urine does not automatically mean that this drug could not be effective in controlling the clinical signs of acute mercury poisoning. In view of the limited experience with acetyl penicillamine in chronic mercurialism, it is difficult to determine at this point:

(1) The efficacy of this drug if used on a long-term basis in removing large amounts of retained mercury,

(2) The effect of this demetallizing on the clinical picture of acute mercury poisoning,

(3) Whether another approach to the problem of mercury removal from the human body should be sought.

Acknowledgments

The postmortem specimen analyses were made by John P. Fahy. Dr. H. V. Aposhian supplied the acetyl-penicillamine used.

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Protection against Sealed Gamma Sources

THE NATIONAL BUREAU OF STANDARDS *Handbook 73, Protection Against Sealed Gamma Sources* is now available at 30 cents per copy from the Superintendent of Documents, U.S. Government Printing Office, Washington 25, D.C. The handbook contains the recommendations of the National Committee on Radiation Protection and Measurements.

One of the primary considerations leading to its preparation as a revision of Handbook 54 has been the modification of the recommendations of the maximum permissible radiation exposure to man. In addition, greater distinction has been made between mandatory recommendations and those which are advisory only. The present Handbook deals with protection against sealed gamma-ray sources and with discrete gamma-emitting sources that are not technically "sealed" but that may be treated in the same manner. The general principles outlined are applicable to all sources of these types. No specific references are made to industrial applications; however, the basic principles and the attenuation data of this Handbook are applicable to both medical and industrial uses.

A Generator for Producing Low Concentrations of Vapor in Inhalation Chambers

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THE inhalation studies being carried out in these laboratories are directed primarily toward determining some of the possible effects of low concentrations of the various known constituents of Los Angeles Basin "smog" upon mammalian organisms. Among the known constituents are various olefin compounds ranging from 4 to 5 carbon atom configurations to 8 or 9 carbon atom configurations. There are other volatile hydrocarbons known or suspected which are also of interest. The studies are planned to use single pure substances such as hexene, octene, etc., or various combinations of these substances with low concentrations of ozone, oxides of nitrogen, carbon monoxide, etc. The concentrations of the volatile hydrocarbons range from fractions of a part per million parts of air, to approximately 15 parts per million of air.

The generation of concentrations of vapors as suggested above, the maintenance of these concentrations over 6–8 hour periods, and the necessity for reproducibility of each concentration day after day, are rather difficult. A review of the literature reveals a number of methods, all ingenious, but none applicable to the conditions imposed in the present studies. It was necessary, therefore, to develop a vapor generator to suit the requirements of low, constant, reproducible concentrations of vapor in air. The generator developed involves no new principles but, rather, is a simplified combination of existing principles. After its successful use in producing constant, low reproducible concentrations of hexene, hour after hour, in the inhalation chambers, it was felt that this device might prove helpful to anyone desiring to produce low to moderately low concentrations of vapors of any volatile liquids in air or any inert carrier gas.

The design of the generator includes control over temperature of the liquid and vapor phase of the substance being vaporized, by means of a water jacket surrounding the device. The design also allows for control of the surface area of the substance exposed for vaporization by means of a tapered volatilization chamber. The flow rate of carrier gas also can be controlled easily. These three controls are moderately sensitive and allow the operator to adjust the amount of material to be vaporized per unit time over a comparatively wide range. A 5 ml burrette, calibrated in 0.02 ml divisions, is incorporated in the construction of the equipment, thus making it possible to read, directly and accurately, the volume of liquid material vaporized per unit time. The inhalation chamber used has a volume of 40 cubic feet. There is a mixing manifold in the air intake pipe. Diffusion cones are placed at the air inlet to the chamber. The air circulating through the chamber is accurately measured by means of a venturi meter in the air intake line. Thus, knowing the volume of intake air per unit time, it is possible, using the volume in milliliters of material vaporized per unit time, to construct a nomograph presenting the parts per million by weight of the material in air within the chamber.

A schematic diagram of the generator and appurtenances, as used in these laboratories, is shown in Figure 1. For control of the temperature of the device, any well controlled water bath should suffice. However, we were fortunate in having a bath in which the temperature could be controlled from slightly above 0°C up to 100°C. The water is circulated through the system by means of a small, electrically driven pump. The problem of clarity of the water in the jacket and its tendency to form air bubbles on the burrette was resolved by the use of a filter, made by filling a one-inch-diameter, foot-long tube with activated charcoal, which was inserted in the water circulating line between the pump and the water jacket.

The carrier gas can be either compressed gas in tanks, such as oil pumped nitrogen, or compressed air supplied from a standard compressor. In the case of the compressed gases, the usual regulator valve will control the flow with a high degree of accuracy but, in the case of the compressed air, it was found necessary to insert a

This investigation was supported by a PHS Research Grant, S-68, from the Bureau of State Services, Public Health Service.

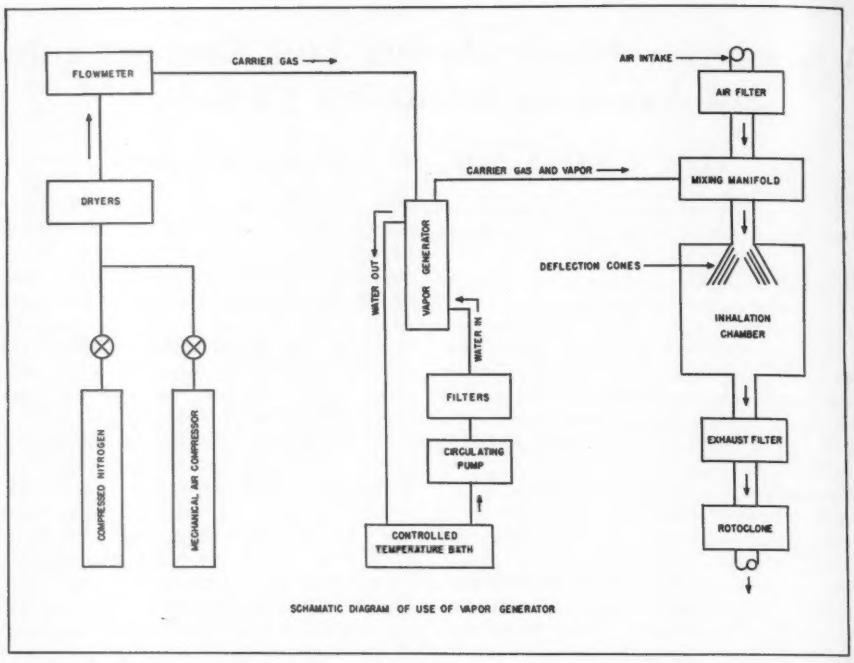

FIGURE 1.

Shrader Air Pressure Regulator in the line to smooth out the variations in pressure. The volume flow of carrier gas may range from about 0.5 liter per minute to 2.0 liter per minute and is measured by means of an all glass flowmeter, such as the Corning Glass Flowmeter #5960. The surface area of volatile liquid to be exposed is increased or decreased by raising or lowering the liquid level in the tapered volatilization chamber (see Figure 2). The accurate control of this level is maintained by use of the principle of the Marriot Bottle. The hypochrome tubing, the bottom end of which sets the level of the liquid in the volatilization chamber, is adjusted up and down by means of a screw thread, and reproducibility of level is easily possible. The liquid level in the volatilization chamber will be slightly lower than the lower end of the hypochrome tubing, due to the slightly over atmospheric pressure produced by the carrier gas. This level will remain constant, however, as long as the flow of carrier gas is not varied.

Since this device is intended for use with all types of volatile liquids, it is suggested that care be taken in the choice of stopcock lubricant used. For example, in the case of hydrocarbons, especially those which are good fat solvents, a stopcock lubricant called "Phynal" (Podbielniak, Inc., Chicago, Illinois) was employed with success.

In checking the accuracy of delivery, constancy of delivery, and reproducibility of the results, a series of volatile liquids was used, ranging from highly volatile compounds to compounds of relatively low volatility. Rather extensive studies were performed on two compounds: n-butyl alcohol and carbon tetrachloride. The results of these studies are presented as evidence of the flexibility of control of the apparatus. The constancy of output and the ease of reproducibility of output are not so evident, but it was found that the measured output varied only insignificantly, hour after hour and the reproducibility of results from day to day was found to be excellent. The over-all accuracy of delivery depends upon the accuracy of control of the three variables: *viz.*, temperature, area of surface exposed for volatilization, and rate of carrier gas flow. The effect of changing these variables can be noted in the accompanying curves (Figure 3). These curves are self-explanatory, except for a word about the term "arbitrary unit" used in the liquid level scale. These "units" were levels of liquid in the tapered

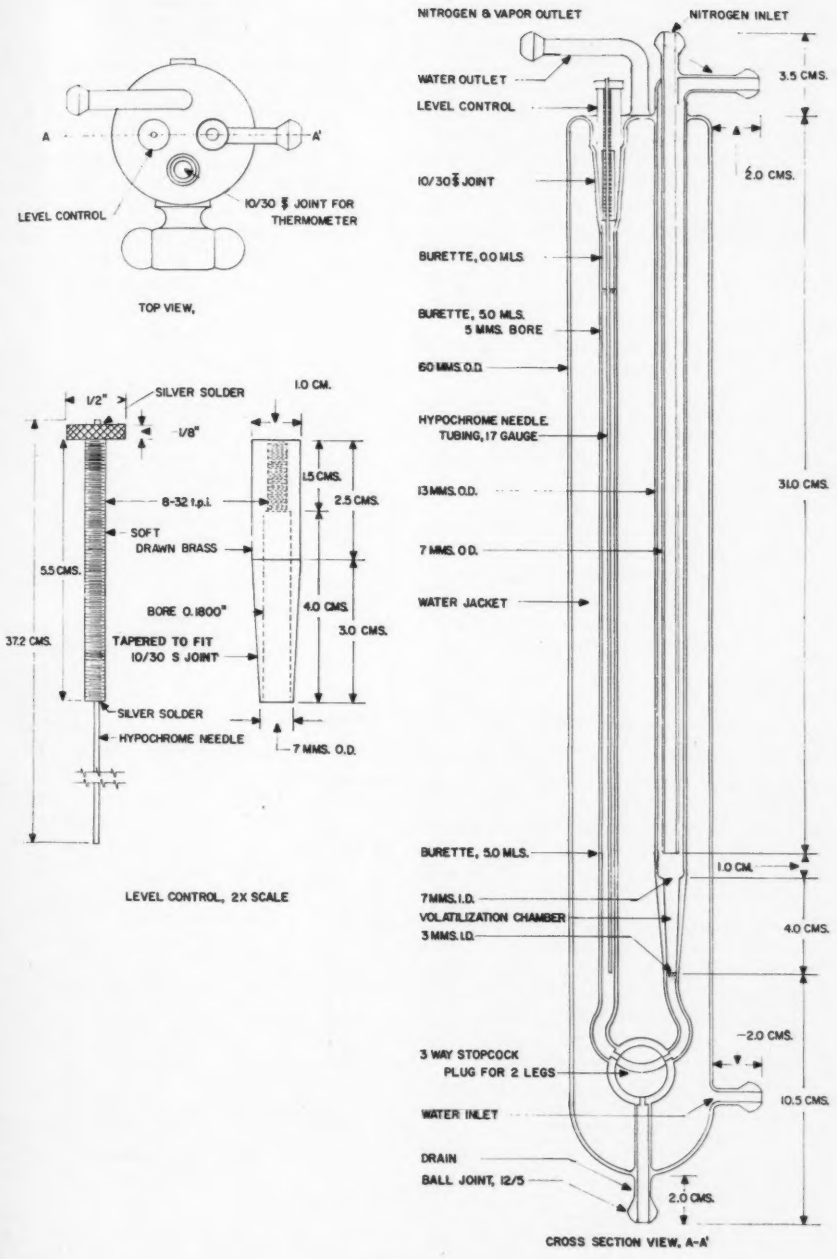
A
A'
LEVEL CONTROL
10/30 S JOINT FOR THERMOMETER
TOP VIEW,
1/2"
SILVER SOLDER
1/8"
1.0 CM.
1.5 CMS.
2.5 CMS.
8-32 t.p.i.
SOFT DRAWN BRASS
5.5 CMS.
BORE 0.1800"
4.0 CMS.
3.0 CMS.
37.2 CMS.
TAPERED TO FIT 10/30 S JOINT
SILVER SOLDER
HYPOCHROME NEEDLE
7 MMS. O.D.
LEVEL CONTROL, 2X SCALE
NITROGEN & VAPOR OUTLET
NITROGEN INLET
WATER OUTLET
LEVEL CONTROL
3.5 CMS.
10/30 S JOINT
2.0 CMS.
BURETTE, 0.0 MLS.
BURETTE, 5.0 MLS. 5 MMS. BORE
60 MMS. O.D.
HYPOCHROME NEEDLE TUBING, 17 GAUGE
13 MMS. O.D.
31.0 CMS.
7 MMS. O.D.
WATER JACKET
BURETTE, 5.0 MLS.
1.0 CM.
7 MMS. I.D.
VOLATILIZATION CHAMBER
3 MMS. I.D.
4.0 CMS.
2.0 CMS.
3 WAY STOPCOCK PLUG FOR 2 LEGS
10.5 CMS.
WATER INLET
DRAIN
BALL JOINT, 12/5
2.0 CMS.
CROSS SECTION VIEW, A-A'

FIGURE 2.

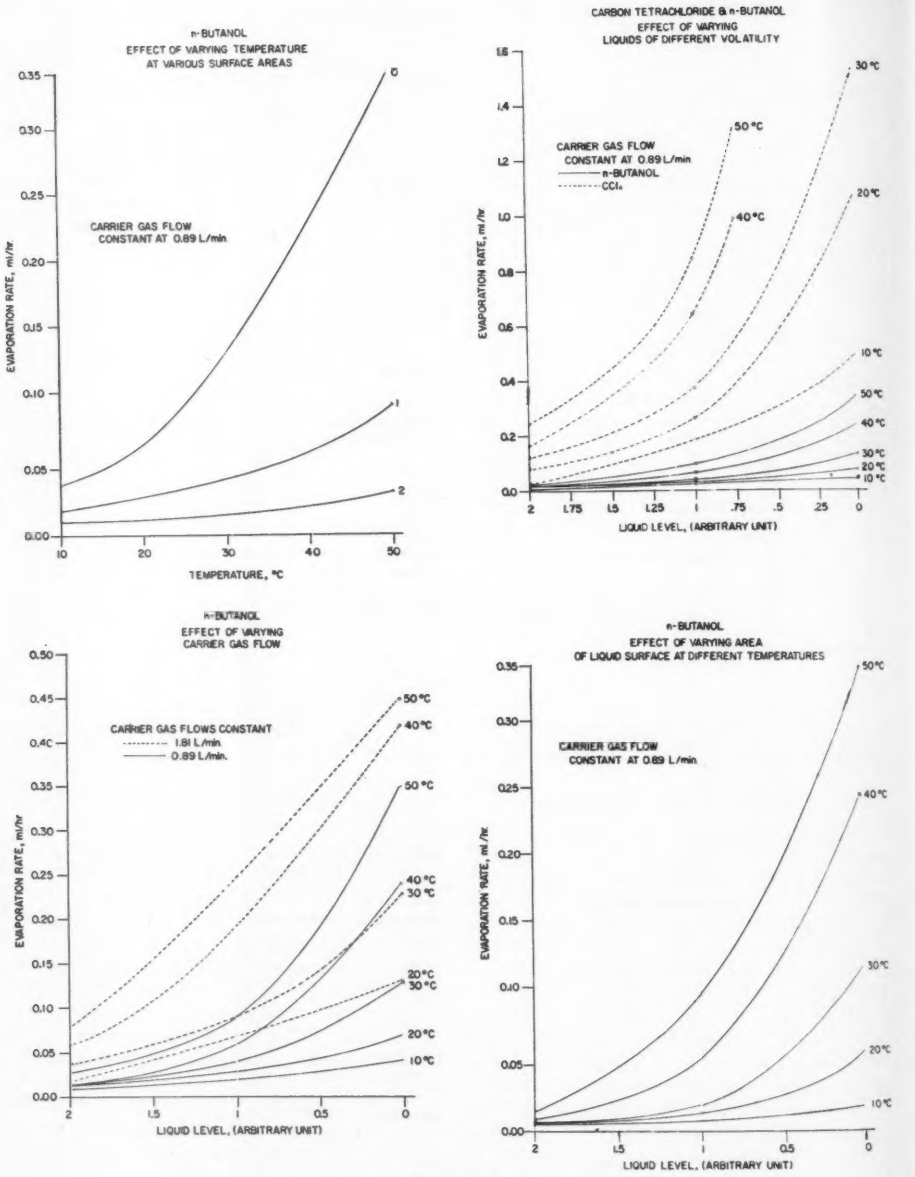

FIGURE 3.

volatilization chamber arbitrarily chosen for reference. Actually, the zero point was the highest level in the chamber which would correspond to the largest surface area. Each unit corresponds to approximately one-quarter of an inch lowering of the liquid level, thus unit 2 would be one-half of an inch below the highest level, and would have a reduced volatilization area dependent upon the taper of the chamber. In none of the runs was it necessary to go beyond

unit 3. These levels were reproducible by means of a fine screw thread adjustment on the hypochrome tubing and it is recognized that the arbitrary level units also could have been expressed as turns of the screw from a "full in" or "full out" position.

A check on the amount of material vaporized into the carrier gas was made on a number of runs done with n-butyl alcohol. These checks were made by analyzing the effluent carrier gas by gas chromotography. The check runs were done at random times in order to secure unbiased results. The findings by gas chromatography were consistently higher by five to six percent than those by direct volume readings. This difference is thought to be due to the additional variables and sources of error inherent in the gas chromotography procedure. However, these checks further demonstrated the constancy of delivery and the reproducibility of results from day to day.

With all volumes corrected to 25°C and 760 mm pressure, it was found that when one change of air per minute was produced in the inhalation chamber, the upper limit of concentration for a material such as carbon tetrachloride was 30 ppm (by weight) and the lower limit around 0.5 ppm (by weight). These values could be doubled, tripled, or even quadrupled by reducing the air change below once per minute and, conversely, could be halved or quartered by increasing the air flow.

Work has been done using other materials, including pure hexene, an olefin known to be present in Los Angeles smog. Results have indicated that the vapor generator operates very satisfactorily with almost any volatile liquid, and its use is suggested where low concentrations are desired.

National Conference on Water Pollution

THE SURGEON GENERAL OF THE U. S. PUBLIC HEALTH SERVICE has called for a National Conference on Water Pollution to be held in Washington, D. C., at the Sheraton-Park Hotel, December 12–14, 1960. The purpose of the Conference will be to assess the problem of water pollution, to determine its effect on our national welfare, and to set realistic goals for its control. The theme of the Conference will be "Clean Water—A Challenge to the Nation."

One interesting feature of the program is a Congressional Roundtable to be addressed by four members of Congress prominently identified with water resources and management. Both houses of the U. S. Congress will be represented; from the Senate will be Robert S. Kerr (Oklahoma) and Francis Case (South Dakota), from the House will be John Blatnik (Minnesota) and William Cramer (Florida). In addition, four sessions of panel discussions will cut across all aspects of the water pollution and quality problems. Outstanding speakers have been chosen for each panel.

This is a citizen's conference reflecting the universal interest of Americans in this essential natural resource. For further information about the Conference write to Frank A. Butrico, Executive Secretary, National Conference on Water Pollution, U. S. Public Health Service, Washington 25, D. C.

Efficiencies of Filter Papers for Collecting Radon Daughters

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THE common method of measuring atmospheric concentrations of radon daughters consists of drawing a measured volume of air through a filter paper and counting the alpha activity on the paper at an appropriate time after sampling. From this activity the concentration of radon daughters can be calculated by the use of correction factors for build-up and decay and the proper calibration factors.[1] The validity of such methods depends on the efficiency of collection of the airborne radioactive particulates, which are either attached to atmospheric dust or are present as free atoms. The investigation reported in this article was undertaken to determine the efficiency of several papers which are commonly used in field methods with particular attention given to aerosol assay membrane paper having an average pore size of 0.80 microns. This paper is the medium we usually employ. Since atmospheric condensation nuclei appear to be the most difficult particles to collect by filtration, efforts were made to test atmospheres containing particulates of primarily sub-micron sizes.

The efficiencies of the filter papers were measured by drawing the test atmospheres through one-inch diameter papers held in a sampling head directly attached to the inlet of an electrostatic precipitator tube. Barnes and Penny,[2] using a Tyndallometer to check the effluent air, reported that the precipitator collected tobacco smoke with practically 100 per cent efficiency and therefore we assumed that this device would collect sub-micron particles such as condensation nuclei. The experimental data of Fraser[3] indicate that in the sub-micron range smaller particles were precipitated more readily than larger ones. In our work the precipitator tube was lined with a thin aluminum insert on which were deposited the particulates which penetrated the filter papers.

The test atmospheres were established in a 61,000-liter sealed chamber. For the first series of tests, this chamber was flushed with Salt Lake City air and then sealed. Sufficient radon was then introduced to give a concentration in the room of about 15,000 $\mu\mu$c per liter. After mixing, the atmosphere was allowed to stand four hours for growth of the radon daughters. Measured volumes of air were then drawn through the filter papers, and the alpha activities on the papers and liners were counted in a proportional counter. For the second series of tests, the air in the chamber was circulated for 1.5 hours through a filter composed of a one-inch thick glass fiber batt in series with a high efficiency asbestos filter. After this treatment, the air again was allowed to stand for four hours.

The total alpha activities on the liners were calculated from the measured activities of a series of 1⅛-inch discs which were punched out of the liner and counted in a proportional counter. The activity on the discs decreased as their distance from the entrance of the tube increased, with no activity above background on the last disc. A flow rate of 10 liters per minute was used in all tests except for the membrane paper. As the performance of this paper with varying flow rates was of particular interest, it was tested at two different rates—10 lpm and 1.3 lpm. Decay curves were run on the filter papers and each disc and the counts were all standardized to a time of 30 minutes after the end of sampling. The shapes of all the decay curves indicated that in both test atmospheres the radon daughters were present in approximately the same equilibrium ratios.

The results of the tests are listed in Table I. The Whatman filter papers which were tested were considerably less efficient in collecting fine particulates than were the glass fiber and membrane papers. The Whatman 40 was considerably more efficient than the Whatman 41, though neither appeared to be highly efficient for collecting fine particulates at low sampling rates. Both the glass fiber and membrane papers showed high efficiencies of collection. The second series of tests, using filtered air and membrane paper only, showed no activity above background on the precipitator liners, thus indicating that the filter was highly efficient for collecting unattached atoms. This is similar to the results reported by Chamberlain and Dyson.[4]

TABLE I

Efficiencies of Various Filter Papers

Filter type	Flow rate liters/ min.	Per cent penetration	Per cent collection efficiency
Atmospheric air			
Whatman 41	10	57.5	42.5
Whatman 40	10	16.2	83.8
Hurlbut Glass Fiber	10	0.297	99.70
Membrane	10	0.034	99.97
Membrane	1.3	0.236	99.76
Filtered air			
Membrane	10	Not detectable	100
Membrane	1.3	" "	100

It should be emphasized that the test atmospheres represented severe conditions that would seldom be encountered in industry. The dust in the unfiltered air was primarily non-settleable, and in the second series of tests all but the very finest particles had been removed. The results of these limited tests indicate that membrane filters are highly efficient in collecting sub-micron particles and that Whatman 41 filters are not highly efficient for collecting sub-micron particles.

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INTERNATIONAL ASBTRACTING AND INFORMATION SERVICE

THE INTERNATIONAL LABOR OFFICE, in cooperation with the International Social Security Association and national health and safety institutions from 23 countries, has established in Geneva, Switzerland a new international abstracting and information service known as CIS—the International Occupational Safety and Health Information Center. Abstracts of the literature appearing in each of these countries are furnished to CIS by the national centers. The abstracts are translated into the three working languages (English, German, and French), coded according to a specially devised classification, and published on 3 x 5 inch cards. At intervals of three to four weeks cards of new abstracts are sent to subscribers; cumulative alphabetic indexes are issued periodically.

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In the United States, the Technical Information Section, Division of Occupational Health, U.S. Public Health Service, Cincinnati, Ohio is serving as the national center for health. The national center for safety is the Division of Safety, Bureau of Labor Standards, U.S. Department of Labor, Washington, D. C.

Statewide Air Pollution Standards in California

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Introduction

CALIFORNIA, as most other states, has traditionally followed the pattern that the responsibility for the control of air pollutants is that of local governmental agencies. In accordance with this pattern, a number of county or regional air pollution control districts have been formed within the State. These districts have provided an effective and satisfactory means for the control of air pollutants from fixed sources.

The recognition that motor vehicles are a major factor in "photochemical smog" indicated a need for a more uniform statewide approach to the control of air pollutants from these mobile sources. Motor vehicles constitute an air pollution factor which is common to all the metropolitan regions of the State. Almost 80 per cent of the people and a slightly higher percentage of the motor vehicles in California are found in the areas where photochemical smog is experienced. Because motor vehicles are a major source of pollutants emitted into the atmosphere of these areas, it is apparent that this type of air pollution cannot be corrected unless present emissions from motor vehicles are reduced. Motor vehicles move freely across the boundaries of local jurisdictions and are licensed and registered by the State. These factors represent a unique situation, which makes local control of this mobile source of air pollutants very difficult, if not impractical. As a result, it was concluded that the State level of government must assume some responsibility for the control of emissions from motor vehicles.

In accepting a broader responsibility for dealing with the problem of air pollution, the State Legislature and the executive branch of the State government concluded that standards for air quality in California communities and for motor vehicle exhaust emissions should be first established. The purpose of standards for air quality was to define the quality of air to be maintained in communities and to establish goals for air pollution control programs. Motor vehicle exhaust emission standards would be based on those for air quality and in turn would be the basis for enactment of specific legislation to control this source of pollutants. Accordingly, the Legislature in early 1959 passed bills requiring the California State Department of Public Health to develop and publish by February 1, 1960, standards for the quality of air and for emission of exhaust contaminants from motor vehicles. These standards were adopted by the State Board of Public Health on December 4, 1959.

Following the establishment of the standards by the Department, the Governor of California called a special legislative session early in 1960 on the subject of air pollution. Both the standards on air quality and motor vehicle exhaust emissions played an important role in the legislation that was considered. A very significant bill was enacted during the special session, requiring air pollution control devices to be installed on motor vehicles. These devices will have to conform to the exhaust standards adopted by the State Department of Public Health.

Local and State Responsibilities in California

The basic roles and responsibilities of local and State levels of government in the solution of air pollution problems in California have been defined by the State Legislature. Its assignment of responsibility has largely determined the nature of activities undertaken in this field by all governmental agencies in California.

The Legislature in 1947 first provided for local air pollution control districts and indicated that these districts had the basic responsibility for the control of emissions to the atmosphere.[1] This acknowledged that the control of air pollution essentially was a matter of local concern and that responsibility for abatement of air pollution should be placed at the lowest level of government capable of dealing with the problem.

In 1955 the Legislature recognized that air pollution had developed to a point where it was a problem of concern to the State as well as to local governmental agencies and provided that the State Department of Public Health maintain a

Presented at the Twenty-first Annual Meeting of the American Industrial Hygiene Association, Rochester, New York, April 27, 1960.

program of air sanitation.[2] This legislative action established the role of the State Department of Public Health primarily to be one of investigation, fact finding, interpretation, coordination and technical assistance. The State was not given the authority to adopt and enforce rules and regulations for the purpose of controlling emissions of pollutants to the atmosphere, as this responsibility has been assigned to air pollution control districts.

By requiring the State Department of Public Health to adopt standards on air quality and motor vehicle exhaust contaminants, the Legislature in 1959 broadened the responsibilities of the State level of government in the field of air pollution. More recently, the State was given specific responsibilities for the control of air pollution when the Legislature enacted a bill requiring control devices on motor vehicles.

Although the State has assumed responsibilities for the control of one important source of pollutants, the basic philosophy regarding air pollution control in California continues to be that air pollution control should be undertaken by the lowest level of government capable of dealing with the problem. Control activities for fixed sources of air pollution are still to be carried out by county and regional air pollution control districts. The State assumed responsibilities for the control of motor vehicle exhaust emission after determining that it was impractical for local agencies to undertake these activities.

Air Quality Standards

The act requiring the State Department of Public Health to set standards on air quality states that "the standards shall be so developed as to reflect the relationship between the intensity and composition of air pollution and the health, illness, including irritation to the senses, and the death of human beings, as well as damage to vegetation and interference with visibility." The air quality standards that were adopted by the State Board of Public Health are given in Table I. Footnotes to the standards were included to make the standards more useful and to present information on other substances that were considered, but for which it was felt there were insufficient data on which to set standards.

The understanding and application of these standards require an appreciation of several factors. In order to take into account the broad range of effects set forth in the legislation, it was necessary that standards include more than one concentration of some substances and more than one effect. As a result, it was decided to establish three levels. These are shown in Table I, which also includes a definition of the effects at each of the levels. The three levels are not merely different degrees of severity but represent qualitatively different effects. For example, some contaminants would first effect health or well-being in the manner defined as occurring at the "serious" level without there being an effect under the "adverse" level. Where the concentration reaches the "serious" or "emergency" levels, different effects may appear rather than just a greater intensity of the phenomena seen at the "adverse" level. Because of the definitions of the three levels and because of the concentrations of pollutants expected to be found in the atmosphere, it would not be necessary to have a concentration for each pollutant under each level. For example, carbon monoxide is not known to produce sensory irritation, damage to vegetation, reduction in visibility or similar effects under the "adverse" level. Instead, the first effects would be expected at the "serious" level. Ethylene is not found in the atmosphere in concentrations that would produce effects as defined under "serious" or "emergency."

Standards were only adopted where it was felt sufficient data permitted a sound judgment to be made. As a result, it was not possible to include many substances which are of concern in the air pollution problems of California communities. The concentrations of pollutants under the three levels were based on biological groups most sensitive to air pollution effects, rather than for the general population. It, therefore, is not expected that persons in good health would experience impairment of bodily function under the concentrations at the "serious" level or acute illness under the concentrations at the "emergency" level. The standards were set for air pollution conditions in California. They cannot be used for all air pollution problems; nor would they have application for industrial workroom exposures.

Most standards that have been adopted represent levels at which no effect is expected. The air quality standards are somewhat unique in that they are concentrations at which first effects are expected. They do not represent a factor of safety or concentrations at which no effect would be experienced.

No legislation has been enacted which makes the air quality standards mandatory or gives them the force of rules and regulations. At present they are recommendations of the Department and have been officially adopted by the State Board of Public Health. The aim in establishing standards was to provide a basis for efforts to control sources of atmospheric pollution. It was important to estimate at what level of pollutants certain specified effects began to occur because some minimum levels of pollutants are unavoid-

TABLE I

Standards for Ambient Air Quality
California State Department of
Public Health

Pollutant	"ADVERSE" LEVEL Level at which there will be sensory irritation, damage to vegetation, reduction in visibility or similar effects	"SERIOUS" LEVEL Level at which there will be alteration of bodily function or which is likely to lead to chronic disease	"EMERGENCY" LEVEL Level at which it is likely that acute sickness or death in sensitive groups of persons will occur
Oxidant	"Oxidant index"	Not applicable	Not applicable
Ozone	0.15 ppm for one hour by the potassium iodide method (eye irritation, plant damage and visibility reduction)	Footnote 1	Footnote 2
Nitrogen Dioxide		Footnote 3	Footnote 3
Hydrocarbons		Footnote 4	Footnote 4
Photochemical Aerosols		Not applicable	Not applicable
Carcinogens	Not applicable	Footnote 5	Not applicable
Sulfur Dioxide	1 ppm for 1 hour or 0.3 ppm for 8 hours (plant damage)	5 ppm for 1 hour (bronchoconstriction in human subjects)	10 ppm for 1 hour severe distress in human subjects)
Sulfuric Acid	Footnote 6	Footnote 6	Footnote 7
Carbon Monoxide	Not applicable	30 ppm for 8 hours or 120 ppm for 1 hour (interference with oxygen transport by blood)	Footnote 8
Lead	Not applicable	Footnote 9	Footnote 9
Ethylene	Footnote 10	Not applicable	Not applicable
Particulates	Sufficient to reduce visibility to less than 3 miles when relative humidity is less than 70 percent	Not applicable	Not applicable

1. Ozone, at one ppm for eight hours daily for about a year, has produced bronchiolitis and fibrositis in rodents (Stokinger, H. E., Wagner, W. D., and Dobrogorski, O. J. *AMA Arch. Ind. Health 16:* 514, 1957). Extrapolation of these data to man is difficult. Functional impairment data have been reported by Clamann and Bancroft (Clamann, H. G., and Bancroft, R. W. *Advances in Chemistry. No. 21,* pp. 352–359, Amer. Chem. Soc., 1959); at 1.25 ppm some effect is observed on residual volume and diffusing capacity. The variability of the tests was not reported. Additional data would be needed before a standard is set.

2. A value of 2.0 ppm of ozone for one hour may produce serious interference with function in healthy persons, and the assumption is made that this might cause acute illness in sensitive persons. (Clamann, H. G., *op cit.*)

TABLE I—*Continued*

3. Five ppm of nitrogen dioxide for eight hours will produce decreased pulmonary function in animals. Slightly more may produce pulmonary fibrosis (Stokinger, personal communication); nitrogen dioxide from air pollution exposures is usually combined with nitric oxide and ozone. More data on human exposures will be needed prior to setting a standard.

4. Hydrocarbons are a group of substances most of which, normally, are toxic only at concentrations in the order of several hundred parts per million. However, a number of hydrocarbons can react photochemically at very low concentrations to produce irritating and toxic substances. Because of the large number of hydrocarbons involved, the complexity of the photochemical reactions, and the reactivity of other compounds such as nitrogen dioxide and ozone, it is not yet possible to establish "serious" and "emergency" levels for hydrocarbons. From the public health standpoint, the concentration of those hydrocarbons which react photochemically should be maintained at or below the level associated with the oxidant index defined in the "adverse" standard.

5. Carcinogens include a few organic compounds such as some polycyclic hydrocarbons, and some metals such as arsenic and chromium. Studies on effects on such substances are currently under way, but there are not sufficient data, at present, to set standards. In the meantime, it is recommended that concentrations of carcinogens in air should be kept as low as possible.

6. A sulfuric acid mist level of 1 mg/m³ with an average particle size of one micron will produce a respiratory response in man. (Amdur, M. O., Silverman, L., and Drinker, P. *AMA Arch. Ind. Hyg. and Occup. Med. 6:* 305, 1952.) It is not possible to generalize from this for all air pollution conditions, because under natural conditions particle size will vary. Only with large droplets would sensory irritation be produced without other physiological effects.

7. A level of 5 mg/m³ of sulfuric acid mist for a few minutes produces coughing and irritation in normal individuals (Amdur, M. O., Silverman, L., and Drinker, P., *op. cit.*). Presumably, it could cause acute illness in sensitive groups of persons in a period of one hour.

8. Given certain assumptions concerning ventilatory rates, acute sickness might result from a carbon monoxide level of 240 ppm for one hour in sensitive groups because of inactivation of ten per cent of the body's hemoglobin. In any event it is clear that when a population exposure limit has been set for carbon monoxide, because of exposures from other sources, community air pollution standards should be based on some fraction of this limit.

9. It is clear that lead levels should be set on the basis of average values for long periods. While data are abundant concerning human response to eight-hours-a-day, five-days-a-week exposures, data are insufficient for the effects of the continuous exposure inherent in community air pollution. While laboratory studies will be pursued with vigor, it becomes very important that local agencies collect data on existing lead levels. Since lead exposures are from multiple sources, community air pollution standards should be based on a portion of the total limit for population exposure.

10. Ethylene causes severe damage to vegetation. Ornamental plants are severely injured by exposures from 0.2 to 0.5 ppm. Tomatoes and fruit are adversely affected at similar levels. Current work is expected to permit a standard to be set within a year.

able. The final use and application of the standards, if they have merit, will develop with time.

Some of the uses to which the air quality standards may be placed already have been indicated. The standards for "oxidant index" and carbon monoxide were the basis for calculating the motor vehicle exhaust emission standards that the De-

partment also was required to adopt. An "oxidant index" of 0.15 ppm as measured by the potassium iodide method ("adverse" level, Table I) is related to the occurrence of "photochemical smog" and has frequently been exceeded in a number of California communities. This value has been associated with the occurrence of slight eye irritation and "oxidant type" vegetation damage in several areas of the State. Carbon monoxide values in excess of 30 ppm for a period of eight hours ("serious" level, Table I) have been measured in the Los Angeles area on a number of occasions over the past few years. Concentrations of oxidant and carbon monoxide in the atmosphere in Los Angeles and data on emissions of carbon monoxide and hydrocarbons were used to estimate the reduction in motor vehicle pollutants that was needed to meet the air quality standards.

The Los Angeles County Air Pollution Control District for several years has had an alert system which established concentrations for four substances—ozone, oxides of nitrogen, sulfur dioxide and carbon monoxide—for the purpose of preventing a possible disaster in Los Angeles County from air pollution. That District recently made use of the Department's air quality standards when it proposed amendment to the alert levels dealing with carbon monoxide to make the alert levels more consistent with the carbon monoxide standard adopted by the Department.[3] The change proposed by the District included a duration of exposure as well as concentration.

During the past year, the Bay Area Air Pollution Control District has been developing regulations on emissions from a large number of fixed sources of air pollutants. It is expected that the proposed regulations will be adopted by the District Board in the near future. In arriving at emission standards for sulfur dioxide, provisions were made for determining compliance by means of designated ground-level concentrations of sulfur dioxide. The ground-level concentrations proposed by the District were very similar to the Department's sulfur dioxide standard at the "adverse" level.[4] The standard for sulfur dioxide set by the Department at the "adverse" level was used by the District in confirming and supporting its proposed regulations on this substance.

The 1960 special session of the State Legislature, in enacting a bill on the control of motor vehicle exhaust emissions, required control devices to be installed on all new vehicles after such devices have been approved. The bill permits each county, however, to determine if the air in the county is polluted by motor vehicle emissions to the extent devices should be required on used vehicles. A portion of the bill states that the board of supervisors of the county shall be guided by the air quality standards adopted by the State Department of Public Health in determining if the air within the county is polluted by emissions from motor vehicles.

Motor Vehicle Exhaust Standards

Emission limits or standards have been used over a long period of time in the control of atmospheric pollutants from fixed sources. Some of the most common of these are the Ringelmann numbers for smoke, weight of particulate matter per unit volume of effluent gases, and sulfur dioxide concentrations in effluent gases. Emission limits had not been applied to motor vehicles for air pollution purposes, with the possible exception of smoke in the exhaust. The motor vehicle exhaust standards set by the State Department of Public Health are the first effort to define limits of emissions from motor vehicles for air pollution purposes.

The legislative act requiring standards for motor vehicle exhaust emissions stated, "It shall be the duty of the State Director of Public Health to determine by February 1, 1960, the maximum allowable standards of exhaust contaminants from motor vehicles which are compatible with the preservation of the public health, including the prevention of irritation to the senses." Standards for exhaust contaminants that were adopted by the State Board of Public Health are shown in Table II.

Before establishing the standards, several basic decisions had to be reached. Because of the wording in the law, it was decided that the standard would be for emissions from the tail pipe and not for other sources of pollutants from vehicles. One standard would be established, based on the region of the State with the most serious air pollution problem, rather than several standards for the different areas of the State. As has already been mentioned in discussing the air quality standards, it was decided that those for motor vehicle exhaust emissions would be based on the air quality standards. In determining the reduction needed in the exhaust emission, the year 1970 was used to estimate total emissions into the atmosphere. The standards had to be designed for a future date since a considerable period of time would be required to place an exhaust control program into effect.

A detailed discussion of the technical data and procedure used to determine the exhaust standards is not possible in this paper. The carbon monoxide presented no particular problem once the air quality standard of 30 ppm for eight hours was established. Considerable data were available on present levels and emissions of carbon monox-

TABLE II

Standards for Motor Vehicle Exhaust Emissions

California State Department of Public Health

The standards of emissions of motor vehicle exhaust contaminants are:[1]

Hydrocarbons—275 parts per million by volume as hexane. (0.165 mole per cent carbon atoms.)

Carbon Monoxide—1.5 per cent by volume.

The standards refer to a composite sample representing the driving cycle described below. Exhaust gas concentrations shall be adjusted to a dry exhaust volume containing 15 per cent by volume of carbon dioxide plus carbon monoxide.

Hydrocarbons are defined as the organic constituents of vehicle exhausts as measured by a hexane-sensitized nondispersive infrared analyzer or by an equivalent method.

Carbon monoxide shall be measured by a nondispersive infrared analyzer or by an equivalent method.

Driving Cycle

Condition	Rate of speed change mph/sec	Percentage of total time	Percentage of total sample volume
Idle	—	15.0	4.2
Cruise			
20 mph	—	6.9	5.0
30 mph	—	5.7	6.1
40 mph	—	2.7	4.2
50 mph	—	0.7	1.5
Acceleration			
0–60 mph	3.0	1.1	5.9
0–25 mph	2.2	10.6	18.5
15–30 mph	1.2	25.0	45.5
Deceleration			
50–20 mph	1.2	10.2	2.9
30–15 mph	1.4	11.8	3.3
30–0 mph	2.5	10.3	2.9
		100.0	100.0

1. It has been judged that an overall 80 per cent reduction of motor vehicle exhaust hydrocarbon emissions is needed. The hydrocarbon concentration expressed in these standards was derived by applying this percentage reduction to the current estimated average exhaust hydrocarbon concentration.

It has been judged that an overall 60 per cent reduction of motor vehicle exhaust carbon monoxide emissions is needed. The carbon monoxide concentration expressed in these standards was derived by applying this percentage reduction to the current estimated average exhaust carbon monoxide concentration.

In the application of the standards to the approval of exhaust control systems, consideration must be given to such factors as warm-up time, reliability and effective life of control devices, and exemption of certain groups of vehicles from compliance. If a substantial number of vehicles are allowed to greatly exceed the standards the objective of the standards may not be reached.

ide and estimated carbon monoxide emissions in Los Angeles in 1970. This estimate indicated that over 90 per cent of the carbon monoxide discharged into the atmosphere of Los Angeles in 1970 would be from motor vehicles. The 60 per cent reduction required in 1970 carbon monoxide emissions to maintain atmospheric concentrations below 30 ppm for eight hours at that time was a direct and simple computation.

The "oxidant index" air quality standard, which was a basis for the hydrocarbon motor vehicle exhaust standard, presented a number of difficulties. The goal of these standards was to reduce "smog" effects to those now experienced when the "oxidant index" is 0.15 ppm or less. Since oxidant and its associated effects are photochemically produced and do not occur in raw exhaust gases, it was necessary to assume some relationships between the "smog" effects and the concentration of exhaust pollutants. The assumption was made that the maximum concentration of eye irritation, "oxidant-type" phytotoxicants and photochemical aerosols which develop in a polluted atmosphere are proportional to the concentration of olefins. Because of the difficulty in measuring olefins and uncertainties about the importance of certain other classes of hydrocarbons, it was decided to express the standard in terms of exhaust hydrocarbons. Two methods were used to estimate the reduction needed in hydrocarbons. One assumed that the above goal could be reached by reducing 1970 olefinic hydrocarbon emissions to the 1940 level in Los Angeles; the second was based on the reduction in hydrocarbons indicated from a study of aerometric data at two stations in the Los Angeles area on severe "smog" days and days when the oxidant was 0.15 ppm. This study was undertaken for the Department by the U.S. Public Health Service. Both methods indicated that approximately 80 per cent of the olefinic hydrocarbons should be eliminated.

The setting of motor vehicle exhaust standards included a number of problems; only some of the most important will be mentioned. Exhaust emissions vary considerably from vehicle to vehicle and under different driving conditions. The several studies of emissions from motor vehicles were different in terms of sampling equipment and driving cycles so that comparisons are uncertain. Methods for hydrocarbon measurements were not considered adequate. The importance of different classes of hydrocarbons and of specific hydrocarbons has not been fully established. The need for nitrogen oxides control, although indicated in a final solution to photochemical air pollution, was not sufficiently established to

permit standards for these compounds at this time.

The motor vehicle exhaust standards have had particular significance in California because they became the basis for legislation to control pollutants from the motor vehicle. As has already been indicated, the State Legislature in March, 1960, passed a bill requiring control devices on motor vehicles.[5] After two or more devices have been approved, a schedule for installation goes into effect, which will require an approved device on all new vehicles sold in the State after one year following the certification date. In addition, the law provides for local option on the requirement of devices on used vehicles over a somewhat longer time period. Before a device can be approved it must meet the motor vehicle exhaust standards that were adopted by the State Department of Public Health.

This recent special session of the Legislature also amended the act giving motor vehicle exhaust emission-setting responsibilities to the State Department of Public Health. The act now permits the Department to adopt standards on all pollutants emitted from motor vehicles. It also allows the Department to take into consideration vegetation damage and interference with visibility in setting these standards.

Conclusion

The California State Department of Public Health has adopted standards for air quality and motor vehicle exhaust emissions. These standards represent a new approach to the air pollution problem in California. Use already has been made of both standards. Those for motor vehicle exhaust have been made the basis for legislation to control pollutants emitted from vehicles.

Standards can only be guides. They cannot be substituted for judgment but must be used intelligently and with the appreciation of their limitations as well as their applications. The standards that were adopted are a first step and will need to be revised and extended as new information becomes available. It is hoped the adoption and use of the standards will stimulate further research into this entire subject.

References

1. *California Health and Safety Code, Section 24199.*
2. *California Health and Safety Code, Sections 425 and 426.*
3. Los Angeles County Air Pollution Control District: *Rules and Regulations,* (1960).
4. Bay Area Air Pollution Control District: *Proposed Regulation No. 2* (1960).
5. *California State Legislature 1960, Assembly Bill No. 17.*

USPHS Division of Air Pollution

THE U. S. PUBLIC HEALTH SERVICE has created a new Division of Air Pollution which will consolidate the research, technical assistance, and training activities of the former Air Pollution Engineering and Medical programs. Chief of the new Division will be Vernon G. MacKenzie, with Dr. Richard A. Prindle as Deputy Chief and Ralph C. Graber as Assistant Chief. Research and other operations will be carried on by five branches: Laboratory of Medical and Biological Sciences, Laboratory of Engineering and Physical Sciences, Field Studies Branch, Research and Training Grants Branch, and Technical Assistance Branch. The new organizational pattern is intended to strengthen and facilitate the efforts of the Public Health Service in the discharge of their research and control activities in the expanding field of air pollution control.

Photofluorographic Study

EDWARD J. BAIER, M.P.H.

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THE photofluorographic unit, known as the chest x-ray unit, has proven to be a valuable tool for the early diagnosis of tuberculosis and allied chest conditions. In principle, a subject is placed between an x-ray tube and a fluoroscopic screen. A camera, mounted behind the screen, photographs the image produced by the x-rays. A photocell, coupled to the x-ray tube, determines the time necessary to obtain the photograph.

Many papers have been written which compare patient and technician exposures to various diagnostic x-ray machines.[1, 2, 3, 4, 5] In all of these, it is concluded that a greater amount of radiation is received by persons in the area of a photofluorographic unit than by other types of x-ray units. In order to determine the magnitude of exposure, a study was made during 1957 and 1958.[6] The parameters of the study at that time included evaluation of the direct beam, exposure at the backs of subjects in the gonadal region, and the exposure of persons in the vicinity of photofluorographic units.

As a result of the initial study a number of recommendations were made. These included the addition of adequate cones to confine the beam, the addition of filters to remove soft x-rays, the use of the operational shield, the positioning of clerks and subjects waiting to be x-rayed, and the positioning of the unit to avoid exposure in occupied areas. To evaluate exposure from these units when all of these precautions were being carried out, the present follow-up study was undertaken.

Method of Study

During both studies new 0–200 milliroentgen dosimeters and a new ionization chamber survey meter were used. The dosimeter readings were corrected by means of charts supplied by the manufacturer for the energy of the x-radiation produced by the units surveyed. The ionization chamber survey meter was calibrated for time response. The dosimeters were used for both subject radiation measurements and for area monitoring; the survey meter was used for area monitoring only.

Presented at the Twenty-first Annual Meeting of the American Industrial Hygiene Association, Rochester, New York, April, 1960.

During the initial study two dosimeters were tied to a cord, and the cord was placed around the subject like a belt. This was done so as to measure the quantity of radiation before and after penetration. During the present study, the same technique was used. It was apparent that no measurable quantity of radiation was being detected after penetration. In order to determine an order of magnitude the same dosimeter was used repeatedly, without charging. After thirty-two exposures the dosimeter indicated one or two milliroentgens. It was assumed that the quantity of radiation after penetration could not be determined with any degree of accuracy, and no further measurements were made. It may be assumed that all radiation entering the body below the lung field was absorbed. The procedure was altered in that only one dosimeter, affixed to a spring clip, was used. It was placed below the area of the direct beam and over the gonadal region to measure the amount of radiation reaching the back of the subject.

Effect of Cones, Diaphragms, and Filtration

The kilovoltage applied to an x-ray tube determines the minimum wave length of the x-rays being produced. The ionization characteristics also change with kilovoltage. The milliamperage determines the number of photons produced and has no effect on the wavelength or the penetrating characteristics of the x-rays. From data obtained by experimentation,[7] the effect of kilovoltage on ionization is presented in Figure 1. Only the inherent filtration, 0.5 millimeters of aluminum equivalent, was present in the direct beam. All milliamperage was calculated to be 130, since most photofluorographic units operate at this current. The calculations included a factor to correct for a 41-inch x-ray tube target-to-screen distance. It may be noted that a direct arithmetical relationship exists between kilovoltage and ionization.

Direct beam intensity varies inversely with the square of the distance. It follows the physical laws of electromagnetic energy. As the distance increases the intensity is distributed over a larger area. Safe practice, therefore, dictates that the area under observation should not be larger than

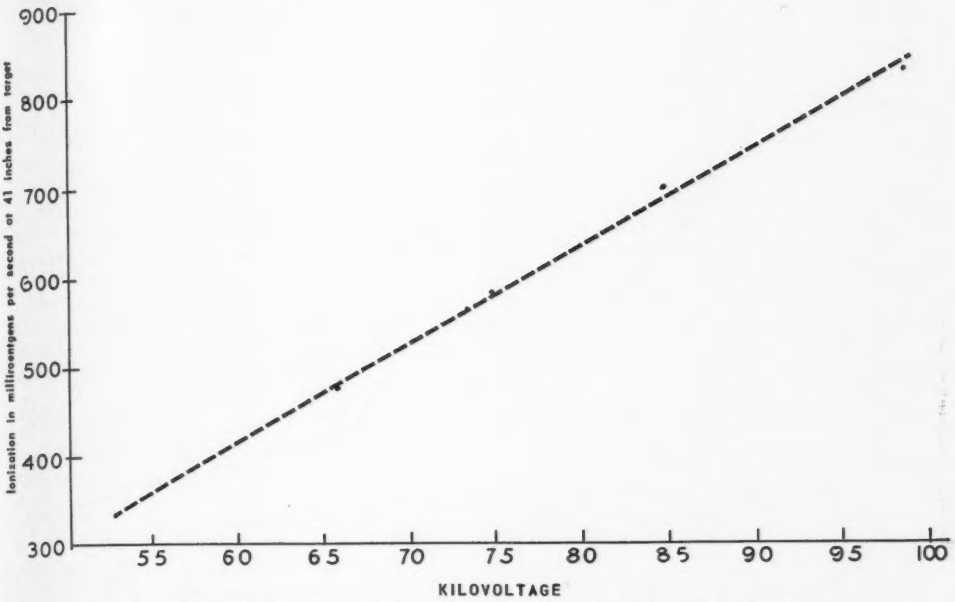

FIGURE 1. Effect of kilovoltage on the production of ionizing radiation (calculations made at 130 ma).

necessary. Lead sheet with an open area or cones to confine the spread of the beam can reduce the total radiation delivered to the subject.

Filtration limits exposure of the subject by removing the long wavelength rays. These soft rays are not capable of passing through body tissue to the fluoroscopic screen. They are principally absorbed by tissue. By placing sheets of aluminum between the x-ray tube target and the subject, the soft rays are removed. The effect of filtration on the reduction of ionizing radiation is demonstrated in Figure 2.[7] All data were calculated from previous experimental studies and corrected so as to conform to the conditions of operation of photofluorographic units. It may be noted from the graphic data that a significant reduction of ionizing radiation results from the installation of aluminum filters.

Procedure

The 0–200 milliroentgen dosimeters used were recently acquired before both studies. These were in good calibration and were energy dependent within 5 per cent from 250 Kev to 1.2 Mev. Since photofluorographic units operate at much lower energy levels, a correction factor was applied. This correction factor was determined to be 0.55.

The ionization chamber survey meter was also new. In order to utilize this meter with short operating time, a time-response curve was drawn. The curve for the particular meter is presented in Figure 3. It may be noted that full response is not reached on this meter in less than three seconds. In order to determine an order of magnitude for area monitoring purposes, the milliampere-second (mas) dial of the photofluorographic unit was recorded for each survey meter observation. The exposure time was found by dividing the mas by the operating milliamperage. These measurements agreed favorably with dosimeters used as area monitors.

Resume of Findings from Initial Study

Dosimeters placed on the backs of approximately 700 subjects to measure the quantities of radiation before penetration indicated that females received a much higher radiation dosage than did males. The mean exposure for all females was 29.4 (range 2.0 to 168.0) milliroentgens per photofluorographic examination. The mean exposure for all males was 11.0 (range 0.0 to 101.0). Although the variance was due in part to the automatic timing device used in the units and in part of the confining qualities of the cones, no explanation could be made for the variance between sex. There was also no apparent correlation between the size of the subject and the dosage.

Technicians and clerical personnel were fre-

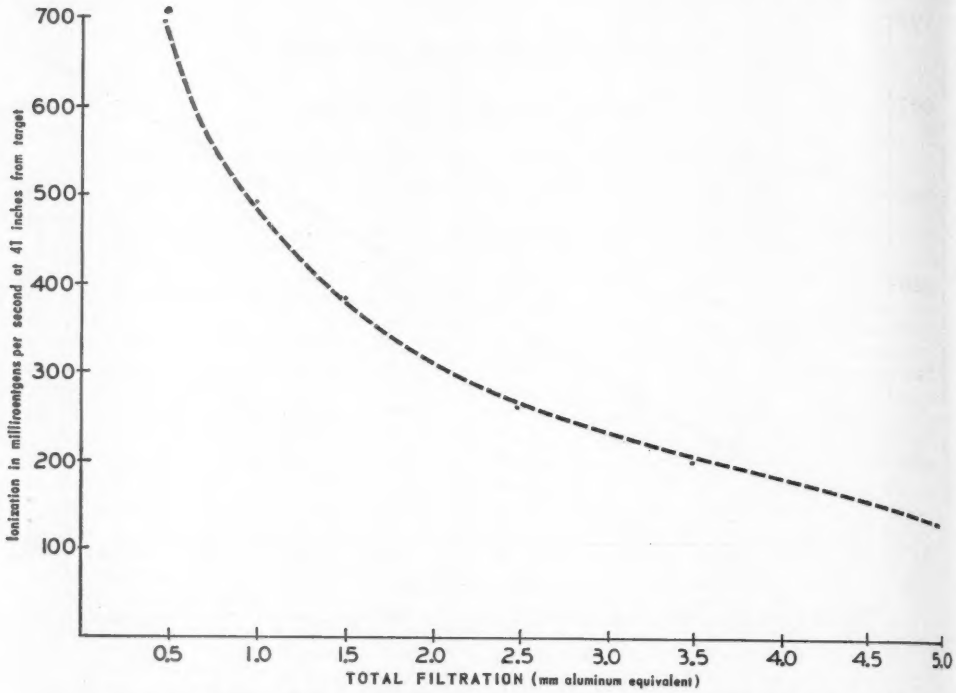

FIGURE 2. Effect of filtration on the reduction of ionizing radiation (calculations made at 85 Kv and 130 ma).

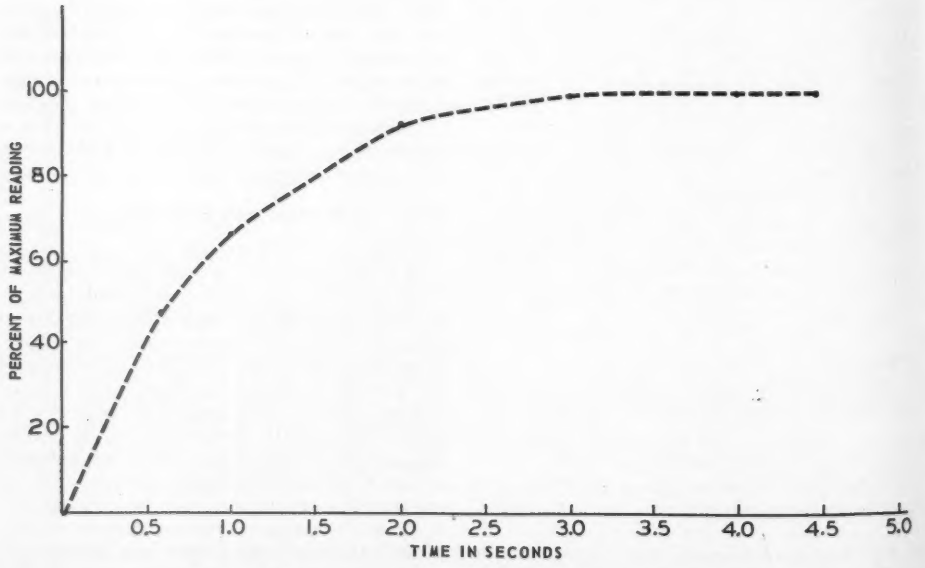

FIGURE 3. Time-response of ionization chamber survey meter.

quently overexposed to ionizing radiation receiving as much as 0.25 to 1.00 milliroentgen per average photofluorographic x-ray. This resulted from the positioning of personnel beyond the protection of the units' operational shields and frequently in the path of scattered radiation generated by the subject being x-rayed and scattered into the area.

Subjects in waiting lines frequently crowded the x-ray unit, approaching the person being x-rayed. In one instance a dosimeter was placed in the shirt pocket of one individual in the line. As he moved in turn through the line his cumulative exposure was determined. He received 18 milliroentgens of unnecessary exposure from the scattered radiation of sixteen subjects.

Follow-up Study

Only photofluorographic units which met certain criteria were studied. These included units with lead cones to confine the x-ray beam to the screen panel and sufficient filtration for the removal of soft, long-length x-rays (filtration of at least 2.5 millimeters aluminum equivalent). In addition, waiting areas were defined and portable lead screen barriers were positioned to protect persons in the area or clerks were positioned more than 25 feet from the unit. Since the use of faster film emulsions had become common practice in these units during the study interim, all units surveyed utilized this type of film.

Subject exposures were determined by means of a dosimeter (described above) placed on the back of the subject in the area just below the lung field. Five hundred and eighty-four determinations were made, 266 male and 318 female. All units surveyed operated at 80 KV and 130 ma. Because of the fast film used by these units, no measurement exceeded the readability of the milliampere dial. The mean exposure time for all persons surveyed was 0.54 seconds. During the initial study the maximum mas dial deflection was exceeded for almost all x-ray exposures.

Since the exposure time was available during the study, a dosage-time distribution could be calculated. Because time was the only variable and exposure varies directly with time, it is conceiva-

TABLE I

Exposures Found at Back of Male Subjects Receiving Photofluorographic Chest X-rays Compared with Time of Exposure

Time (seconds)	Milliroentgens per examination	
	average dosage	range
0.3	2.56	1.0–5.0
0.4	3.24	1.0–6.0
0.5	4.61	1.0–20.0
0.6	6.15	2.0–12.0
0.7	6.36	1.0–10.0
0.8	8.73	1.0–18.0
0.9	12.34	6.0–23.0
1.0		
1.1		
1.2	13.00	

TABLE II

Exposures Found at Back of Female Subjects Receiving Photofluorographic Chest X-rays Compared with Time of Exposure

Time (seconds)	Milliroentgens per examination	
	average dosage	range
0.3	6.6	1.0–12.0
0.4	8.7	2.0–29.0
0.5	12.6	2.0–52.0
0.6	17.1	7.0–32.0
0.7	19.0	12.0–28.0
0.8	27.6	10.0–55.0
0.9	31.0	15.0–47.0
1.0	34.0	27.0–41.0
1.1	36.8	12.0–43.0
1.2	70.0	46.0–121.0

TABLE III

Stray Radiation Levels Determined at a Photofluorographic Unit Installation

Location	Amount of Stray Radiation (milliroentgens per 100 exposures)
At control panel of unit	1.05
Five feet behind control panel of unit	0.87
Over top of unit's operational shield	20.50
Beyond side of unit's operational shield	21.06
Clerks' positions, twenty-five feet from left side of unit	6.25
Through lead glass of portable lead barrier to protect subjects in waiting line	0.35
Beyond side of portable lead barrier	5.55

TABLE IV

Comparison of Exposures Found at Point of Measurement of Persons Receiving Photofluorographic Chest X-ray

Point of Measurement	Milliroentgens per average photofluorographic examination	
	Dosage for initial study	Dosage for follow-up study
Back of male subjects, before penetration	11.0	5.29
Back of female subjects, before penetration	29.4	16.30

ble that a direct arithmetic proportion would be found. This is true for male subjects, and the data are presented in Table I. For 0.3 seconds exposure, the average dosage was 2.56 milliroentgens; for 0.6 seconds, 6.15 milliroentgens; and for 0.9 seconds, 12.34 milliroentgens. Within experimental error, the exposure progressed linearly with time. This relationship was not exhibited with female subjects. Data for the female are presented in Table II. To use the same time factors as for the male, for 0.3 seconds exposure the average dosage was 6.6 milliroentgens; for 0.6 seconds, 17.1 milliroentgens; and for 0.9 seconds, 31 milliroentgens. These data do not follow a similar progression, and in all instances are between 2.5 and 2.8 times the male dosage for a given exposure time. Possibly the differences in bone and muscle structure between the sexes account for a difference in exposure.

When a photofluorographic unit is positioned in such a manner that persons in waiting lines and clerical personnel are protected by distance or shielding, a great amount of unnecessary radiation exposure may be avoided. Ionizing radiation levels measured at an installation are reported in Table III. Significant reductions were noted as compared with those found during the initial study. These data are presented in Table III. Clerks positioned twenty-five feet from the unit received a dosage slightly more than 6 milliroentgens per 100 photofluorographic examinations. Those placed behind portable lead barriers received approximately one-third milliroentgen per 100 exposures. A person in the waiting line who could not crowd the unit because of a clearly defined waiting area received only one milliroentgen during his wait. This was approximately 5 per cent of the same exposure recorded during the initial study.

Comparison of Gonadal Exposure

Table IV contains a tabulation comparing average ionizing radiation exposures found at the backs of male and female subjects during conditions when no confining qualities or filtration are added to the x-ray beam, and when cones and filters are added. In general, a reduction of 50 per cent was found. This is a significant reduction, since all unnecessary radiation is to be avoided.

Summary

A comparison study was made between photofluorographic units. Initially, units with only inherent filtration and large cones were studied. Exposures of subjects, technicians, and clerical personnel were evaluated. During a follow-up study, only units which met certain criteria were evaluated. Significant reduction of exposures was noted, and the exposures at the backs of subjects in the gonadal region were reduced 50 per cent. Tables and graphs indicating the effects of kilovoltage and milliamperage are presented.

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Air Pollution Course

A COURSE IN AIR POLLUTION will be offered by the New York University Post-Graduate Medical School and the College of Engineering, November 28 through December 9, 1960. This two-week intensive course is designed for engineers, physicians, industrial hygienists, and others concerned with the problem of community air pollution. Topics to be covered are: Economic Factors, Toxic Emissions, Micrometeorology, Applications of Meteorology, Surveys, Sampling and Analysis, Air Cleaning, Power Plants, Control in Specific Industries, Effects on Vegetation, Government Regulations, and Public Relations. A field trip to Brookhaven National Laboratory is planned. Tuition is $100 and the field trip will cost $10. Address inquiries to Office of Associate Dean, New York University Post-Graduate Medical School, 550 First Avenue, New York 16, N.Y.

News and Notes

Personnel Notes

Andrew D. Hosey and Alfred C. Mendenhall, Jr. received the Michigan (Detroit) Section Award for the outstanding paper in the 1959 volume of the AIHA Journal. Their paper was "The Envirec—An Instrument for Continuous Recordings of Environmental Air, Wet-Bulb, and Globe Temperatures, and Air Velocity." Presentation of the award was made by Paul M. Giever, President of the Michigan Industrial Hygiene Society.

Robert J. Halen, M.D. has been appointed Medical Director for Jones & Laughlin Steel Corporation effective June 1, 1960.

Charles E. Couchman has retired as Director of the Bureau of Industrial Hygiene, Baltimore City Health Department and is now affiliated with the U.S. Atomic Energy Commission, State Relations Group, Office of Health and Safety, Germantown, Maryland.

Gilbert B. Meyers, M.D., formerly with the Radio Corporation of America has accepted a position as Medical Director, Mobil Oil Company, Paulsboro, New Jersey.

Robert Pring has left American Wheelabrator and is now Manager, Dust Collector Sales, Dracco Division of Fuller Co.

Dr. Allen D. Brandt has been appointed as Manager of a newly established Department of Industrial Health Engineering in the Bethlehem Steel Company. He will coordinate and guide the company-wide programs in industrial hygiene, air pollution control, water pollution control, noise control, radiation protection, and related activities.

Obituary

Constantin P. Yaglou died at his home in Belmont, Massachusetts on June 3, 1960. He was born in Constantinople (Istanbul), Turkey, and came to the United States about 1919. Cornell University conferred the M.M.E. degree on him in 1921 and he went to Harvard School of Public Health where he served continuously until his death. Best known for his teaching and research in climatic adaptation, he contributed greatly also to the field of industrial hygiene engineering. His membership in AIHA dated from 1940, shortly after formation of the Association. He was also a member of APHA, ASHVE, AAAS, Sigma Xi and Delta Omega. The passing of a man of the professional and scientific stature of Constantin Yaglou is indeed a loss to industrial hygiene.

Laboratory for Hearing Research

THE UNIVERSITY OF MICHIGAN MEDICAL CENTER has announced plans to construct the world's largest medical laboratory devoted exclusively to research on hearing. The facility will be known as the Kresge Hearing Research Institute and is expected to be completed by 1963.

Special equipment such as anechoic and reverberation chambers, vibrationless platforms, electronic laboratories, shielded rooms, tape-recording facilities, glassworking shop, and testing and examining rooms will be available to the researchers. Extensive work will be done with patients. A multidisciplinary attack will seek to further our knowledge of the mechanism and the functioning of the complex organs of hearing, as well as preventive and therapeutic measures in hearing loss. Seven departments of the Medical School will expand their research into hearing and its problems.

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——Carbon Disulfide
——Carbon Monoxide
——Carbon Tetrachloride
——Chlorine Dioxide
——Chromic Acid
——Cresol
——DDT
——Diborane
——1,2-Dichloroethane
——Diethylamine
——Diethylene Triamine
——Dimethylformamide
——Dinitrophenol
——Epoxy Resin Systems
——Ethyl Alcohol
——Ethyl Benzene
——Ethylenediamine
——Ethylene Dibromide
——Ethylene Glycol Monomethyl Ether
——Ethylene Glycol Mono-n-Butyl Ether
——Ethylene Oxide
——Fluoride-Bearing Dusts and Fumes
——Fluorine
——Formaldehyde
——Heptane
——Hexane
——Hydrogen Chloride
——Hydrogen Cyanide
——Hydrogen Fluoride
——Hydrogen Peroxide
——Hydrogen Selenide
——Hydrogen Sulfide
——Hydrazine
——Lead and its Inorganic Compounds
——Magnesium
——Mercury
——Meta-dinitrobenzene
——Metal Hydride
——Methyl Alcohol
——Methyl Bromide
——Methyl Ethyl Ketone
——Methylene Dichloride
——Molybdenum
——Nickel Carbonyl
——Nitrobenzene
——Nitrogen Dioxide
——Nitroglycerin
——Nitropropane
——Ozone
——Parathion
——Pentaborane
——Pentachlorophenol and Sodium Pentachlorophenate
——Phenol
——Phosphoric Acid
——Phosphoric Anhydride
——Polonium
——Proplyene Oxide
——Pyridine
——Radon and Daughters
——Selenium and Compounds
——Silica (Amorphous)
——Silica (Free Silica, Silicon Dioxide)
——Sodium Chlorate
——Styrene Monomer
——Sulfur Dioxide
——Sulfuric Acid
——Teflon, tetrafluoroethylenes
——Tetrachlorethylene
——Tetrahydrofuran
——Thorium and Compounds
——Titanium
——Toluene
——Toluene 2,4 Diisocyanate
——Trichloroethylene
——1,1,1-Trichloroethane
——Uranium (Natural)
——Vanadium Pentoxide
——Xylene
——Zinc Oxide
——Zirconium

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